

09/044696

(FILE 'USPAT' ENTERED AT 15:31:41 ON 27 JUL 1999)

L1 1085 SEA (ADP(W)RIBOSYLAT? OR CHOLER? OR PERTUSS? OR COLI) (3W)
TOXIN# OR (CT OR PT OR LT) (10A)TOXIN#
L2 9 SEA L1 AND ((DETOX? OR DE(W)TOX?) (5A) (MUTAT? OR MUTAGEN?
OR MUTANT#))

=> d 1-9 .bevpatt

US PAT NO: 5,856,122 [IMAGE AVAILABLE] L2: 1 of 9
TITLE: Modification of pertussis toxin
DATE ISSUED: Jan. 5, 1999
INVENTOR: Randy J. Read, Edmonton, Canada
Penelope E. Stein, Edmonton, Canada
Stephen A. Cockle, Richmond Hill, Canada
Raymond P. Oomen, Tottenham, Canada
Sheena Loosmore, Aurora, Canada
Michel H. Klein, Willowdale, Canada
Glen D. Armstrong, Edmonton, Canada
Bart Hazes, Edmonton, Canada
SEARCH-FLD: 435/15, 69.1

ABSTRACT:

The three-dimensional structure of crystalline pertussis holotoxin (PT) has been determined by X-ray crystallography. Crystal structures have also been determined for complexes of pertussis toxin with molecules relevant to the biological activity of PT. These three-dimensional structures were analyzed to identify functional amino acids appropriate for modification to alter the biological properties of PT. Similar procedures may be used to predict amino acids which contribute to the toxicity of the holotoxin, to produce immunoprotective, genetically-detoxified analogs of pertussis toxin.

US PAT NO: 5,849,530 [IMAGE AVAILABLE] L2: 2 of 9
TITLE: Manipulation of gene copy number in bordetella
DATE ISSUED: Dec. 15, 1998
INVENTOR: Sheena Loosmore, 70 Crawford Rose Drive, Aurora, Ontario,
Canada, L4G 4R4
Gavin Zealey, 348 Charlton Avenue, Thornhill, Ontario,
Canada, L4J 6H7
Reza Yacoob, 2354 Old Pheasant Road, Mississauga, Ontario,
Canada, L5A 2S1
Michel Klein, 16 Munro Boulevard, Willowdale, Ontario,
Canada, M2P 1B9
SEARCH-FLD: 435/69.3, 69.1, 172.3, 243, 252.3, 320.1; 424/234.1,
235.1, 240.1, 184.1, 192.1; 536/23.1, 23.2, 23.7

ABSTRACT:

A protein expression levels from Bordetella strains, particularly Bordetella pertussis, are altered by genetic modification to a natural Bordetella strain whereby one or more of the natural genes, particularly
Searcher : Shears 308-4994

including the TOX, FHA, CYA and PRN genes, is deleted from the genome of the natural strain and one or more of the natural genes or a genetic **mutation** thereof, particularly a genetically-detoxified TOX* gene, or a hybrid gene, is inserted into the genome of the natural strain to provide at least two copies of one or more of the natural genes or genetic mutation thereof or hybrid gene, singly or in tandem. The altered genotype Bordetella strain is useful in producing whole-cell or defined component vaccines against Bordetella, particularly whooping cough, which may be employed in combination with other vaccines.

US PAT NO: 5,439,810 [IMAGE AVAILABLE] L2: 3 of 9
 TITLE: Manipulation of gene copy number in bordetella
 DATE ISSUED: Aug. 8, 1995
 INVENTOR: Sheena Loosmore, Aurora, Canada
 Gavin Zealey, Thornhill, Canada
 Reza Yacoob, Mississauga, Canada
 Michel Klein, Willowdale, Canada
 SEARCH-FLD: 424/88, 92, 234.1, 235.1, 240.1; 435/69.1, 172.3, 243,
 69.3, 320.1, 252.3; 935/38; 536/27, 23.1, 23.2, 23.7

ABSTRACT:

Protein expression levels from Bordetella strains, particularly Bordetella pertussis, are altered by genetic modification to a natural Bordetella strain whereby one or more of the natural genes, particularly including the TOX, FHA, CYA and PRN genes, is deleted from the genome of the natural strain and one or more of the natural genes or a genetic **mutation** thereof, particularly a genetically-detoxified TOX* gene, or a hybrid gene, is inserted into the genome of the natural strain to provide at least two copies of one or more of the natural genes or genetic mutation thereof or hybrid gene, singly or in tandem. The altered genotype Bordetella strain is useful in producing whole-cell or defined component vaccines against Bordetella, particularly whooping cough, which may be employed in combination with other vaccines.

US PAT NO: 5,433,945 [IMAGE AVAILABLE] L2: 4 of 9
 TITLE: Immunoprotective genetically-detoxified mutants of
 pertussis toxin
 DATE ISSUED: Jul. 18, 1995
 INVENTOR: Michel H. Klein, Willowdale, Canada
 Heather A. Boux, Aurora, Canada
 Stephen A. Cockle, Richmond Hill, Canada
 Sheena M. Loosmore, Aurora, Canada
 Gavin R. Zealey, Concord, Canada
 SEARCH-FLD: 424/88, 92, 93, 94; 435/252.3; 530/350, 351, 387, 388,
 403, 405, 406

ABSTRACT:

A new method is described for the preparation of a safe, immunogenic and efficacious vaccine for protection against the disease pertussis. In development of this vaccine, specific functional sites of **pertussis toxin** have been identified, and using this information, defined

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mutant holotoxins have been produced by site directed mutagenesis of the toxin gene. A number of these holotoxin analogues are detoxified, retain an immunodominant S1 epitope, are immunogenic and are protective in the standard pertussis vaccine potency test in mice.

US PAT NO: 5,358,868 [IMAGE AVAILABLE] L2: 5 of 9
 TITLE: Genetic detoxification of **pertussis toxin**
 DATE ISSUED: Oct. 25, 1994
 INVENTOR: Michel H. Klein, Willowdale, Canada
 Heather A. Boux, Aurora, Canada
 Stephen A. Cockle, Richmond Hill, Canada
 Sheena M. Loosmore, Aurora, Canada
 Gavin R. Zealey, Concord, Canada
 SEARCH-FLD: 435/69.1, 69.3, 252.1, 172.1, 172.2, 172.3, 243; 536/27,
 23.5; 935/10, 11, 12, 65; 530/324, 350

ABSTRACT:

A new method is described for the preparation of a safe, immunogenic and efficacious vaccine for protection against the disease pertussis. In development of this vaccine, specific functional sites of **pertussis toxin** have been identified, and using this information, defined mutant holotoxins have been produced by site directed mutagenesis of the toxin gene. A number of these holotoxin analogues are detoxified, retain an immunodominant S1 epitope, are immunogenic and are protective in the standard pertussis vaccine potency test in mice.

US PAT NO: 5,332,583 [IMAGE AVAILABLE] L2: 6 of 9
 TITLE: Vaccine containing genetically-detoxified pertussis
 holotoxin
 DATE ISSUED: Jul. 26, 1994
 INVENTOR: Michel H. Klein, Willowdale, Canada
 Heather A. Boux, Aurora, Canada
 Stephen A. Cockle, Richmond Hill, Canada
 Sheena M. Loosmore, Aurora, Canada
 Gavin R. Zealey, Concord, Canada
 SEARCH-FLD: 435/69.7, 252.3, 68.1, 193, 194, 252.4, 253.6, 252.1,
 71.2, 243, 248, 832; 424/92, 88; 530/403-406, 350, 387

ABSTRACT:

A new method is described for the preparation of a safe, immunogenic and efficacious vaccine for protection against the disease pertussis. In development of this vaccine, specific functional sites of **pertussis toxin** have been identified, and using this information, defined mutant holotoxins have been produced by site directed mutagenesis of the toxin gene. A number of these holotoxin analogues are detoxified, retain an immunodominant S1 epitope, are immunogenic and are protective in the standard pertussis vaccine potency test in mice.

US PAT NO: 5,244,657 [IMAGE AVAILABLE] L2: 7 of 9
 TITLE: Genetic detoxification of **pertussis toxin**
 DATE ISSUED: Sep. 14, 1993
 INVENTOR: Michel H. Klein, Willowdale, Canada

Searcher : Shears 308-4994

Heather A. Boux, Aurora, Canada
 Stephen A. Cockle, Richmond Hill, Canada
 Sheena M. Loosmore, Aurora, Canada
 Gavin R. Zealey, Concord, Canada

SEARCH-FLD: 424/88, 92

ABSTRACT:

A new method is described for the preparation of a safe, immunogenic and efficacious vaccine for protection against the disease pertussis. In development of this vaccine, specific functional sites of **pertussis toxin** have been identified, and using this information, defined mutant holotoxins have been produced by site directed mutagenesis of the toxin gene. A number of these holotoxin analogs are detoxified, retain an immunodominant S1 epitope, are immunogenic and are protective in the standard pertussis vaccine potency test in mice.

US PAT NO: 5,221,618 [IMAGE AVAILABLE] L2: 8 of 9

TITLE: Genetic detoxification of **pertussis toxin**

DATE ISSUED: Jun. 22, 1993

INVENTOR: Michel H. Klein, Willowdale, Canada
 Heather A. Boux, Aurora, Canada
 Stephen A. Cockle, Richmond Hill, Canada
 Sheena M. Loosmore, Aurora, Canada
 Gavin R. Zealey, Concord, Canada

SEARCH-FLD: 435/69.1, 69.3, 252.1, 172.3; 536/27; 935/10, 11, 12, 65

ABSTRACT:

A new method is described for the preparation of a safe, immunogenic and efficacious vaccine for protection against the disease pertussis. In development of this vaccine, specific functional sites of **pertussis toxin** have been identified, and using this information, defined mutant holotoxins have been produced by site directed mutagenesis of the toxin gene. A number of these toxin analogues are detoxified, retain an immunodominant S1 epitope, are immunogenic and are protective in the standard pertussis vaccine potency test in mice.

US PAT NO: 5,085,862 [IMAGE AVAILABLE] L2: 9 of 9

TITLE: Genetic detoxification of **pertussis toxin**

DATE ISSUED: Feb. 4, 1992

INVENTOR: Michel H. Klein, Willowdale, Canada
 Heather A. Boux, Aurora, Canada
 Stephen A. Cockle, Richmond Hill, Canada
 Sheena M. Loosmore, Aurora, Canada
 Gavin R. Zealey, Concord, Canada

SEARCH-FLD: 424/92; 435/252.3; 530/350, 387, 403, 405, 406

ABSTRACT:

A new method is described for the preparation of a safe, immunogenic and efficacious vaccine for protection against the disease pertussis. In development of this vaccine, specific functional sites of **pertussis toxin** have been identified, and using this information, defined

Searcher : Shears 308-4994

mutant holotoxins have been produced by site directed mutagenesis of the toxin gene. A number of these toxin analogues are detoxified, retain an immunodominant S1 epitope, are immunogenic and are protective in the standard pertussis vaccine potency test in mice.

=> d his 13-; d 1-5 .bevpat

(FILE 'USPAT' ENTERED AT 15:31:41 ON 27 JUL 1999)

L3 1 S BARCHFELD, G?/IN
 L4 0 S (DELGIUDICE, G? OR DEL GIUDICE, G?)/IN
 L5 10 S RAPPUOLI, R?/IN
 L6 0 S L3 AND L5
 L7 5 S (L3 OR L5) AND L1
 L8 5 S L7 NOT L2

- Author(s)

US PAT NO: 5,925,546 [IMAGE AVAILABLE] L8: 1 of 5
 TITLE: Immunologically active polypeptides with altered toxicity
 useful for the preparation of an antipertussis vaccine
 DATE ISSUED: Jul. 20, 1999
 INVENTOR: Mariagrazia Pizza, Siena, Italy
 Antonella Bartoloni, Siena, Italy
 Rino Rappuoli, Siena, Italy
 SEARCH-FLD: 530/350; 424/240.1, 190.1, 254.1, 832; 435/69.1, 69.3,
 172.3, 320.1; 536/23.7

ABSTRACT:

Immunological active polypeptides with no or reduced toxicity useful for the preparation of an antipertussis vaccine. Method for the preparation of said polypeptides which comprises, cultivating a microorganism transformed with a hybrid plasmid including the gene/s which codes for at least one of said polypeptides in a suitable medium and recovering the desired polypeptide from the cells or from the culture medium.

US PAT NO: 5,908,825 [IMAGE AVAILABLE] L8: 2 of 5
 TITLE: Dosage composition for nasal delivery and method of use of
 the same
 DATE ISSUED: Jun. 1, 1999
 INVENTOR: Alessio Fasano, Ellicott City, MD
 Teresa De Magistris, Siena, Italy
 Sergio Uzzau, Sassari, Italy
 Rino Rappuoli, Querciegrossa, Italy
 SEARCH-FLD: 514/2, 3, 12, 15, 4, 866; 424/261.1, 130.1, 184.1;
 530/303, 362, 351, 387.1, 399

ABSTRACT:

A nasal dosage composition for nasal delivery comprising (A) a therapeutic agent; and (B) zonula occludens toxin, as well as a method for the use of the same.

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US PAT NO: 5,889,172 [IMAGE AVAILABLE] L8: 3 of 5
 TITLE: DNA sequences for immunologically active peptides of
 pertussis toxin
 DATE ISSUED: Mar. 30, 1999
 INVENTOR: Mariagrazia Pizza, Siena, Italy
 Antonella Bartoloni, Siena, Italy
 Rino Rappuoli, Siena, Italy
 SEARCH-FLD: 536/23.7; 435/320.1, 69.1, 69.3, 172.3; 424/190.1, 254.1,
 833

ABSTRACT:

Immunologically active polypeptides with no or reduced toxicity useful for the preparation of an antipertussis vaccine. Method for the preparation of said polypeptides which comprises, cultivating a microorganism transformed with a hybrid plasmid including the gene/s which codes for at least one of said polypeptides in a suitable medium and recovering the desired polypeptide from the cells or from the culture medium.

US PAT NO: 5,785,971 [IMAGE AVAILABLE] L8: 4 of 5
 TITLE: **Pertussis toxin** and use in vaccines
 DATE ISSUED: Jul. 28, 1998
 INVENTOR: **Rino Rappuoli**, Quercegrossa-Monteriggioni, Italy
 Alfredo Nicosia, Siena, Italy
 Maria Beatrice Arico, Quercegrossa, Italy
 SEARCH-FLD: 530/350; 514/12; 424/190.1, 240.1, 254.1; 435/69.1, 193

ABSTRACT:

Cloning and sequencing of the Eco RI fragment of B. pertussis chromosomal DNA with 4696 base pairs, containing the genes which code for the five subunits of the **pertussis toxin**.

A hybrid plasmid containing the DNA fragment or its further fragments and a micro-organism transformed by the hybrid plasmid and capable of expressing the cloned DNA fragment or further fragments thereof by synthesis of the **pertussis toxin** or one or more subunits of the **pertussis toxin**.

The **pertussis toxin** or one or more subunits of the **pertussis toxin** so obtained are useful for the preparation of vaccines and diagnostic kits.

US PAT NO: 5,427,788 [IMAGE AVAILABLE] L8: 5 of 5
 TITLE: **Pertussis toxin** and use in vaccines
 DATE ISSUED: Jun. 27, 1995
 INVENTOR: **Rino Rappuoli**, Quercegrossa-Monteriggioni, Italy
 Alfredo Nicosia, Siena, Italy
 Maria B. Arico', Quercegrossa, Italy
 SEARCH-FLD: 514/12; 435/193, 69.1; 424/190.1, 240.1, 254.1

ABSTRACT:

Cloning and sequencing of the Eco RI fragment of B. pertussis chromosomal DNA with 4696 base pairs, containing the genes which code for the five

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subunits of the **pertussis toxin**.

A hybrid plasmid containing the DNA fragment or its further fragments and a micro-organism transformed by the hybrid plasmid and capable of expressing the cloned DNA fragment or further fragments thereof by synthesis of the **pertussis toxin** or one or more subunits of the **pertussis toxin**.

The **pertussis toxin** or one or more subunits of the **pertussis toxin** so obtained are useful for the preparation of vaccines and diagnostic kits.

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Searcher : Shears 308-4994

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Set	Items	Description
S1	7644	(ADP(W)RIBOSYLAT? OR CHOLER? OR PERTUSS? OR COLI) (3W)TOXIN? ? OR (CT OR PT OR LT) (10N)TOXIN? ?
S2	4	S1 AND ((DETOX? OR DE(W) (TOXIF? OR TOX????)) (5N) (MUTANT? ? OR MUTAGEN? OR MUTAT?))
S3	4	RD (unique items)

? t 3/3,ab/1-4

-key terms

>>>No matching display code(s) found in file(s): 65

3/3,AB/1 (Item 1 from file: 144)

DIALOG(R)File 144:PASCAL

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13712203 PASCAL No.: 98-0403290

Mucosal immunogenicity of genetically detoxified derivatives of heat labile toxin from Escherichia coli

DOUCE G; GIULIANI M M; GIANNELLI V; PIZZA M G; RAPPUOLI R; DOUGAN G

Department of Biochemistry, Imperial College of Science, Technology and Medicine, Exhibition Road, London SW7 2AY, United Kingdom; The Chiron Vaccines Immunological Research Institute, Via Fiorentina 1, Siena 53100, Italy

Journal: Vaccine, 1998, 16 (11-12) 1065-1073

Language: English

Using a fixed dose of antigen, the immune response to detoxified mutants of LT-WT following intranasal (i.n.), subcutaneous (s.c.) and oral (i.g.) immunisation has been studied. When given i.n., both LT-WT and mutant toxin, K63, generated significant levels of toxin-specific IgG in the serum, and the levels of IgA in nasal and lung lavages were greater than those induced by rLT-B. In comparison, i.g. immunisation of mice with a similar quantity of either LT-WT or K63 toxin induced barely detectable levels of IgG in the sera. However,

Searcher : Shears 308-4994

if the amount of protein used for i.g. immunisation was increased tenfold, relatively good levels of toxin-specific IgG were induced in the sera by both LT-WT or K63. Low levels of toxin-specific IgA were also observed in intestinal washes from these mice. Western blotting of the sera, using the native toxin as an antigen, demonstrated the presence of both anti-A and anti-B subunit antibodies. Most significantly, toxin-neutralising antibodies were induced in the serum, with the strongest activity being induced by the LT-WT, an intermediate activity induced by mutant K63 and a lower response by rLT-B. Together, these data show that ADP-ribosyltransferase is not necessary for mucosal immunogenicity of these proteins, and that the i.n. route of immunisation is more effective than the i.g. route of immunisation for the generation of both systemic (IgG) and mucosal (IgA) immune responses.

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3/3,AB/2 (Item 2 from file: 144)
 DIALOG(R) File 144:PASCAL
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11785230 PASCAL No.: 94-0662718

Etude de quatre facteurs de virulence de Bordetella pertussis dans un modele murin d'infection respiratoire

(Study of four virulence factors of Bordetella pertussis by using a murine model of respiratory infection)

KHELEF Nadia; GUIISO Nicole, dir

Universite de Paris 11, Francee

Univ.: Universite de Paris 11. FRA Degree: Th. doct.

1994-06; 1994 240 p.

Language: French Summary Language: French; English

Bordetella pertussis, l'agent de la coqueluche, synthetise plusieurs facteurs de virulence, parmi lesquels deux adhesines, la pertactine et l'hemagglutinine filamenteuse, et deux toxines, la toxine de pertussis et l'adenylcyclase-hemolysine. Leur role a ete etudie a l'aide de mutants n'exprimant pas l'un de ces facteurs dans un modele murin d'infection respiratoire, au niveau de la colonisation et des lesions pulmonaires. Nous avons montre que chacune des adhesines pouvait compenser l'absence de l'autre, mais qu'elles avaient un role dans la persistance bacterienne, suggerant l'existence d'une cooperation entre ces adhesines. A l'inverse, l'adenylcyclase-hemolysine est indispensable a la multiplication bacterienne initiale, alors que la toxine de pertussis est importante en fin d'infection. Les mutants depourvus d'une des toxines induisent peu ou pas d'inflammation pulmonaire, indiquant que ces toxines participeraient a cet effet, soit directement, soit indirectement, en inactivant les cellules immunitaires et en facilitant ainsi l'action d'autres facteurs proinflammatoires. L'etude des interactions cellulaires in vitro a revele que B. pertussis etait cytotoxique pour les macrophages murins qui meurent par apoptose ou mort cellulaire programme. Le mutant depourvu

Searcher : Shears 308-4994

de toxine de pertussis est aussi actif que la souche sauvage, alors que celui qui n'exprime pas d'adenylcyclase-hemolysine n'est plus toxique, suggerant un role pour l'adenylcyclase-hemolysine dans l'apoptose des macrophages. Enfin, nous avons mis en evidence que, malgre leur efficacite contre une infection respiratoire murine par B. pertussis, aucun des quatre facteurs synthetises par B. pertussis ne protegeait contre une infection par Bordetella parapertussis, l'autre espece pathogene pour l'homme, suggerant que cette protection serait specifique d'espece

3/3,AB/3 (Item 3 from file: 144)
DIALOG(R)File 144:PASCAL
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10652240 PASCAL No.: 93-0161524
Progress towards the development of new vaccines against whooping cough
RAPPUOLI R; PODDA A; PIZZA M; COVACCI A; BARTOLONI A; DE MAGISTRIS M T;
NENCIONI L
Immunobiology resz. inst. Siena, 53100 Siena, Italy
Journal: Vaccine, 1992, 10 (14) 1027-1032
Language: English
Acellular vaccines against whooping cough are in the final stage of clinical testing and are likely to become available for mass immunization in the near future. Over a dozen vaccines of similar composition have been developed by vaccine companies and research laboratories; all of them contain a detoxified form of pertussis toxin (PT) that may be present alone or combined with one or more other non-toxic proteins, such as filamentous haemagglutinin (FHA), pertactin (69 kDa), and the agglutinogens (AGG)

3/3,AB/4 (Item 4 from file: 144)
DIALOG(R)File 144:PASCAL
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08820365 PASCAL No.: 89-0369737
BREVET. Genetic detoxification of pertussis toxin
CONNAUGHT LABORATORIES LTD
Publication Date: 1989-06-28
Patent: EP 0322115 A2 Patent Filing: 88311133.8, 1988-11-24
Convention: GB 8727489, 1987-11-24 IPC: C 12N 15/00
Language: English

Set	Items	Description	Author(s)
S4	11	AU=(BARCHFELD, G? OR BARCHFELD G?)	
S5	27	AU=(DELGIUDICE, G? OR DELGIUDICE G? OR DEL GUIDICE, G? OR - DEL GUIDICE G?)	
S6	207	AU=(RAPPUOLI, R? OR RAPPUOLI R?)	
S7	0	S4 AND S5 AND S6	

Searcher : Shears 308-4994

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S8 1 S4 AND (S6 OR S5)
S9 0 S5 AND S6
S10 1 S8 NOT S2
? t 10/3,ab/1

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10/3,AB/1 (Item 1 from file: 144)
DIALOG(R)File 144:PASCAL
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14003751 PASCAL No.: 99-0188851

The adjuvants MF59 and LT-K63 enhance the mucosal and systemic immunogenicity of subunit influenza vaccine administered intranasally in mice

BARCHFELD G L; HESSLER A L; CHEN M; PIZZA M; RAPPUOLI R; VAN NEST G A

Chiron Corporation, 4560 Horton Street, Emeryville, CA 94608-2916, United States; Chiron Vaccines, Via Fiorentina 1, 53100 Siena, Italy

Journal: Vaccine, 1999, 17 (7-8) 695-704

Language: English

Commercial influenza vaccines generate serum antibody, but not local IgA. Influenza vaccines that induce both serum and secretory antibody are more likely to protect against infection and disease progression. The adjuvants MF59 and LT-K63 were tested intramuscularly and intranasally with subunit HA. In naive mice, intranasal adjuvant effect was more apparent when included with the first than second immunization. In previously infected mice, intranasal adjuvants had little effect on serum antibodies and were most effective for nasal antibodies after the second immunization. Overall, both adjuvants enhanced anti-HA IgA and IgG by intranasal vaccination whereas, by intramuscular vaccination, they only enhanced serum IgG.

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Dev, S.
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(FILE 'CAPLUS' ENTERED AT 15:14:03 ON 27 JUL 1999)

-key terms

=> d que

L1 15389 SEA FILE=CAPLUS ABB=ON PLU=ON (ADP(W)RIBOSYLAT? OR
CHOLER? OR PERTUSS? OR COLI) (3W)TOXIN OR (CT OR PT OR
LT) (S)TOXIN
L3 15 SEA FILE=CAPLUS ABB=ON PLU=ON L1 AND (DETOX? OR DE
TOX?) (5A) (MUTANT OR MUTAGEN? OR MUTAT?)

=> d 1-15 .bevstr1

L3 ANSWER 1 OF 15 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1998:672490 CAPLUS
DOCUMENT NUMBER: 129:289177
TITLE: **Detoxified mutants of
bacterial ADP-ribosylating
toxins as parenteral adjuvants**
INVENTOR(S): Barchfeld, Gail; Del Giudice, Giuseppe;
Rappuoli, Rino
PATENT ASSIGNEE(S): Chiron Corporation, USA
SOURCE: PCT Int. Appl., 51 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9842375	A1	19981001	WO 98-US5454	19980319
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9865713	A1	19981020	AU 98-65713	19980319
PRIORITY APPLN. INFO.:			US 97-41227	19970321
			US 98-44696	19980318
			WO 98-US5454	19980319
AB The present invention provides parenteral adjuvants comprising detoxified mutants of bacterial ADP- ribosylating toxins, esp. pertussis toxin (PT), cholera toxin (CT), and Escherichia coli-derived heat-labile Searcher : Shears 308-4994				

toxin (LT). The immune adjuvant includes LT-K63, LT-R72, CT-S109 and PT-K9/G129. LT-K63 was prepd. as parenteral adjuvant for vaccine comprising herpes simplex virus type 2 gD antigen, influenza hemagglutinin, and HIV p24 gag.

IT Diphtheria toxin

RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(CRM197; **detoxified mutants** of bacterial
ADP-ribosylating toxins as parenteral
adjuvants)

IT Heat labile enterotoxin

RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(*Escherichia coli*; **detoxified mutants** of
bacterial **ADP-ribosylating toxins**
as parenteral adjuvants)

IT Toxins

RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(bacterial ADP-ribosylating; **detoxified mutants**
of bacterial **ADP-ribosylating toxins**
as parenteral adjuvants)

IT Adjuvants (immunological)

Bacteria (Eubacteria)
Escherichia coli
Human herpesvirus 2
Human immunodeficiency virus
Influenza
Intramuscular injections
Parenteral solutions (drug delivery systems)
Protein sequences
Subcutaneous injections
Topical drug delivery systems
Vaccines
Vibrio cholerae
(**detoxified mutants** of bacterial ADP
-ribosylating toxins as parenteral adjuvants)

IT Antigens

Cholera toxin
Glycoprotein D
Hemagglutinins
Pertussis toxin
p24 (gag protein)
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(**detoxified mutants** of bacterial ADP
-ribosylating toxins as parenteral adjuvants)

IT Drug delivery systems

(transcutaneous; **detoxified mutants** of
bacterial **ADP-ribosylating toxins**
as parenteral adjuvants)

IT Vertebrate (Vertebrata)

(vaccine; **detoxified mutants** of bacterial

**ADP-ribosylating toxins as parenteral
adjuvants)**

IT 214068-46-9 214068-50-5 214068-55-0

RL: PRP (Properties)
(amino acid sequence; **detoxified mutants** of
bacterial **ADP-ribosylating toxins**
as parenteral adjuvants)

L3 ANSWER 2 OF 15 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1998:476941 CAPLUS

DOCUMENT NUMBER: 129:243790

TITLE: Mucosal immunogenicity of genetically detoxified
derivatives of heat labile toxin from
Escherichia coli

AUTHOR(S): Douce, Gill; Giuliani, Marzia Monica; Giannelli,
Valentina; Pizza, Maria Grazia; Rappuoli, Rino;
Dougan, Gordon

CORPORATE SOURCE: Department of Biochemistry, Imperial College of
Science, Technology and Medicine, London, SW7
2AY, UK

SOURCE: Vaccine (1998), 16(11/12), 1065-1073
CODEN: VACCDE; ISSN: 0264-410X

PUBLISHER: Elsevier Science Ltd.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Using a fixed dose of antigen, the immune response to
detoxified mutants of LT-WT following intranasal
(i.n.), s.c. and oral (i.g.) immunization has been studied. When
given i.n., both LT-WT and mutant **toxin**, K63,
generated significant levels of **toxin**-specific IgG in the
serum, and the levels of IgA in nasal and lung lavages were greater
than those induced by rLT-B. In comparison, i.g. immunization of
mice with a similar quantity of either LT-WT or K63
toxin induced barely detectable levels of IgG in the sera.
However, if the amt. of protein used for i.g. immunization was
increased tenfold, relatively good levels of **toxin**
-specific IgG were induced in the sera by both LT-WT or
K63. Low levels of **toxin**-specific IgA were also obsd. in intestinal
washes from these mice. Western blotting of the sera, using the
native **toxin** as an antigen, demonstrated the presence of both anti-A
and anti-B subunit antibodies. Most significantly, **toxin**
-neutralizing antibodies were induced in the serum, with the
strongest activity being induced by the LT-WT, an
intermediate activity induced by mutant K63 and a lower response by
rLT-B. Together, these data show that ADP-ribosyltransferase is not
necessary for mucosal immunogenicity of these proteins, and that the
i.n. route of immunization is more effective than the i.g. route of
immunization for the generation of both systemic (IgG) and mucosal
(IgA) immune responses.

Searcher : Shears 308-4994

IT Toxins
 RL: BAC (Biological activity or effector, except adverse); BIOL
 (Biological study)
 (heat labile; mucosal immunogenicity of genetically detoxified
 derivs. of heat labile toxin from Escherichia coli)

IT Vaccination
 (intranasal; mucosal immunogenicity of genetically detoxified
 derivs. of heat labile toxin from Escherichia coli)

IT Lung
 (lavage; mucosal immunogenicity of genetically detoxified derivs.
 of heat labile toxin from Escherichia coli)

IT Escherichia coli
 Mucosal immunity
 Nasal mucosa
 Serum (blood)
 (mucosal immunogenicity of genetically detoxified derivs. of heat
 labile toxin from Escherichia coli)

IT IgA
 IgG
 RL: MFM (Metabolic formation); BIOL (Biological study); FORM
 (Formation, nonpreparative)
 (mucosal immunogenicity of genetically detoxified derivs. of heat
 labile toxin from Escherichia coli)

IT Immunization
 (oral; mucosal immunogenicity of genetically detoxified derivs.
 of heat labile toxin from Escherichia coli)

L3 ANSWER 3 OF 15 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1998:337192 CAPLUS

DOCUMENT NUMBER: 129:107991

TITLE: **Pertussis toxin potentiates**
 Th1 and Th2 responses to co-injected antigen:
 adjuvant action is associated with enhanced
 regulatory cytokine production and expression of
 the co-stimulatory molecules B7-1, B7-2 and CD28

AUTHOR(S): Ryan, Mark; Mccarthy, Leone; Rappuoli, Rino;
 Mahon, Bernard P.; Mills, Kingston H. G.

CORPORATE SOURCE: Infection and Immunity Group, Department of
 Biology, National University of Ireland,
 Kildare, Ire.

SOURCE: Int. Immunol. (1998), 10(5), 651-662
 CODEN: INIMEN; ISSN: 0953-8178

PUBLISHER: Oxford University Press

DOCUMENT TYPE: Journal

LANGUAGE: English

AB **Pertussis toxin (PT)** is a major
 virulence factor of Bordetella pertussis which exerts a range of
 effects on the immune system, including the enhancement of IgE, IgA
 and IgG prodn., delayed-type hypersensitivity reactions, and the

Searcher : Shears 308-4994

induction of exptl. autoimmune diseases. However, the mechanism by which PT mediates adjuvant activity remains to be defined. In this investigation the authors have shown that PT can potentiate antigen-specific T cell proliferation and the secretion of IFN- γ , IL-2, IL-4 and IL-5 when injected with foreign antigens. A chem. detoxified PT and a genetic mutant with substitutions/deletions in the S-1 and B oligomer components that abrogate enzymic and binding activity displayed no adjuvant properties. In contrast, a non-toxic S-1 mutant devoid of enzymic activity but still capable of receptor binding retained its adjuvant activity, augmenting the activation of both Th1 and Th2 subpopulations of T cells. To address the mechanism of T cell activation, the authors found that PT stimulated the prodn. of IFN- and IL-2 by naive T cells and IL-1 by macrophages. Therefore potentiation of distinct T cell subpopulations may have resulted in part from the pos. influence of IFN- γ on the development of Th1 cells and the co-stimulatory role of IL-1 for Th2 cells. Furthermore, PT augmented expression of the co-stimulatory mols. B7-1 and B7-2 on macrophages and B cells, and CD28 on T cells, suggesting that the adjuvant effect may also be assocd. with facilitation of the second signal required for maximal T cell activation. This study demonstrates that the immunopotentiating properties of PT are largely independent of ADP-ribosyltransferase activity, but are dependent on receptor binding activity and appear to involve enhanced activation of T cells.

- IT Th1 cell
- Th2 cell
 - (adjuvant activity of pertussis toxin for)
- IT B cell (lymphocyte)
- Macrophage
 - (adjuvant activity of pertussis toxin in relation to induced costimulatory mol. expression by accessory cell)
- IT CD28 (antigen)
- Interferon γ .
- Interleukin 2
- Interleukin 4
- Interleukin 5
- RL: MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative)
 - (adjuvant activity of pertussis toxin in relation to induced expression by T-cell for)
- IT CD80 (antigen)
- CD86 (antigen)
- Interleukin 1
- RL: MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative)
 - (adjuvant activity of pertussis toxin in

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relation to induced expression by accessory cell for)

IT Immunostimulation
(cellular; costimulatory mol. and regulatory cytokine expression
in adjuvant activity of **pertussis toxin**)

IT T cell activation
(costimulatory mol. and regulatory cytokine expression in
adjuvant activity of **pertussis toxin**)

IT **Pertussis toxin**
RL: BAC (Biological activity or effector, except adverse); PRP
(Properties); BIOL (Biological study)
(mechanism of adjuvant activity of)

L3 ANSWER 4 OF 15 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1998:293623 CAPLUS
DOCUMENT NUMBER: 128:279567
TITLE: Immunogenic **detoxified mutant**
Escherichia coli LT-A

toxin

INVENTOR(S): Pizza, Mariagrazia; Giuliani, Marzia Monica;
Rappuoli, Rino
PATENT ASSIGNEE(S): Chiron S.P.A., Italy; Pizza, Mariagrazia;
Giuliani, Marzia Monica; Rappuoli, Rino
SOURCE: PCT Int. Appl., 69 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9818928	A1	19980507	WO 97-IB1440	19971030
W: CA, JP, US				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				

PRIORITY APPLN. INFO.: GB 96-22660 19961031

AB An immunogenic detoxified protein is provided which comprises the amino acid sequence of subunit A of an E. coli heat labile **toxin (LT-A)** or a fragment thereof in which at least amino acid Ala-72 of the A subunit is mutated, preferably by substitution with Arg. The toxoid is useful as vaccine against an enterotoxigenic strain of E. coli and is produced by recombinant DNA means by site-directed mutagenesis. A 1.5 kb SmaI-EcoRI fragment from plasmid pEWD299 contg. the gene for LT-A and the LT promoter region was subcloned to produce vector BS-LT-A. BS-LT-A was mutagenized with oligonucleotide oligoLT-A72R to change the Ala-72 codon to the Arg codon and ligated to the EcoRI-HindIII fragment contg. the gene for LT-B and cloned to produce vector BS-LTA72R. E. coli was transformed with BS-LTA72R and the LT-A72R mutant purified.

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ADP-ribosylation of LT-A72R was lower than wild type LT-A, the toxicity of LT-A72R was 10-5 lower than wild type LT-A, and LT-A72R proved to be an effective mucosal adjuvant.

- IT Adjuvants (immunological)
Detoxification (metabolic)
 Escherichia coli
 Genetic engineering
 Site directed **mutagenesis**
 Vaccines
 (immunogenic **detoxified mutant Escherichia coli LT-A toxin**)
- IT Antigens
 Heat labile enterotoxin
 RL: BPN (Biosynthetic preparation); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
 (immunogenic **detoxified mutant Escherichia coli LT-A toxin**)
- IT 56-41-7DP, Alanine, residue-72
 RL: BPN (Biosynthetic preparation); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
 (immunogenic **detoxified mutant Escherichia coli LT-A toxin** with Ala-72 mutated by substitution)
- IT 74-79-3DP, Arginine, residue-72
 RL: BPN (Biosynthetic preparation); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
 (immunogenic **detoxified mutant Escherichia coli LT-A toxin** with Ala-72 mutated by substitution with Arg)

L3 ANSWER 5 OF 15 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1998:198478 CAPLUS

DOCUMENT NUMBER: 128:307201

TITLE: Recent advances in immunological adjuvants: the development of particulate antigen delivery systems

AUTHOR(S): O'hagan, Derek T.

CORPORATE SOURCE: Chiron Corporation, Emeryville, CA, 94704, USA

SOURCE: Expert Opin. Invest. Drugs (1998), 7(3), 349-359
 CODEN: EOIDER; ISSN: 1354-3784

PUBLISHER: Ashley Publications

DOCUMENT TYPE: Journal; General Review

LANGUAGE: English

AB A review with 70 refs. New generation vaccines, including those based on recombinant proteins, are safer than traditional vaccines, but are less immunogenic. Therefore, there is an urgent need for

Searcher : Shears 308-4994

the development of new and improved vaccine adjuvants. A no. of potent immunostimulatory mols. obtained from bacterial cells or plants have been extensively evaluated as adjuvants. However, a no. of these mols. have displayed significant toxicity, both in preclin. animal models and in human clin. trials. An alternative approach to the development of novel adjuvants involves the prepn. of particulate antigen delivery systems of similar dimensions to natural pathogens. In the absence of addnl. immunostimulatory mols., emulsion droplets and microparticles have been shown to be potent adjuvants for the induction of both humoral and cell-mediated immune responses following systemic administration. Moreover, particulate delivery systems have been shown to display an acceptable toxicity profile in a no. of clin. trials. Particulate antigen delivery systems also have the potential to function as potent adjuvants following administration by mucosal routes, including oral and intranasal. An alternative approach to the mucosal delivery of vaccines involves the use of genetically **detoxified mutant toxins**, e.g., **LT-K63**, as mucosal adjuvants. The use of novel adjuvants and antigen delivery systems is likely to extend the use of vaccines into the area of therapeutics, involving the eradication of infectious diseases and cancers, or the amelioration of autoimmune disorders.

IT Adjuvants (immunological)
Drug delivery systems
Vaccines

(recent advances in immunol. adjuvants and the development of particulate antigen delivery systems)

IT Antigens

RL: BAC (Biological activity or effector, except adverse); THU (Therapeutic use); BIOL (Biological study); USES (Uses)

(recent advances in immunol. adjuvants and the development of particulate antigen delivery systems)

L3 ANSWER 6 OF 15 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1997:557657 CAPLUS

DOCUMENT NUMBER: 127:219543

TITLE: Immunogenic **detoxified mutants**
of **cholera toxin**

INVENTOR(S): Fontana, Maria Rita; Pizza, Mariagrazia;
Rappuoli, Rino

PATENT ASSIGNEE(S): Chiron S.P.A., Italy; Fontana, Maria Rita;
Pizza, Mariagrazia; Rappuoli, Rino

SOURCE: PCT Int. Appl., 53 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

Searcher : Shears 308-4994

09/044696

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9729771	A1	19970821	WO 97-IB183	19970217
W: CA, JP, US				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
CA 2244800	AA	19970821	CA 97-2244800	19970217
EP 880361	A1	19981202	EP 97-902552	19970217
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
PRIORITY APPLN. INFO.:			GB 96-3314	19960216
			WO 97-IB183	19970217

AB An immunogenic detoxified protein comprising the amino acid sequence of subunit A of a **cholera toxin** or a fragment thereof in which at least one amino acid is substituted with another amino acid characterized in that, in purified form, the immunogenic detoxified protein exhibits a residual toxicity greater than 10000 fold lower than its naturally occurring counterpart. In the described embodiment, the amino acid at, or in a position corresponding to Pro-106 is replaced with another amino acid. The immunogenic detoxified protein is useful as vaccine for *Vibrio cholerae* and is produced by recombinant DNA means by site-directed mutagenesis. Vaccine comprises the detoxified **cholera toxin** and a second antigen are also disclosed.

IT **Cholera toxin**
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses) (detoxified; immunogenic detoxified mutants of cholera toxin)

IT DNA
RL: BSU (Biological study, unclassified); BIOL (Biological study) (encoding detoxified cholera toxin; immunogenic detoxified mutants of cholera toxin)

IT Vaccines
Vibrio cholerae (immunogenic detoxified mutants of cholera toxin)

IT Antigens
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses) (immunogenic detoxified mutants of cholera toxin)

L3 ANSWER 7 OF 15 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1995:801623 CAPLUS
DOCUMENT NUMBER: 123:196584
TITLE: Non-toxic mucosal adjuvant
INVENTOR(S): Rappuoli, Rino
PATENT ASSIGNEE(S): Biocine S.p.A., Italy
Searcher : Shears 308-4994

SOURCE: PCT Int. Appl., 29 pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9517211	A1	19950629	WO 95-IB13	19941222
W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ				
RW: KE, MW, SD, SZ, AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
CA 2179771	AA	19950629	CA 94-2179771	19941222
AU 9512785	A1	19950710	AU 95-12785	19941222
EP 732937	A1	19960925	EP 95-903889	19941222
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				

PRIORITY APPLN. INFO.: GB 93-26174 19931222
 WO 94-IB68 19940324
 WO 95-IB13 19941222

AB A non-toxic mucosal adjuvant is provided which may be admixed with further antigens to provide a vaccine administrable to mucosal surfaces in organisms including man. Preferably, the non-toxic mucosal adjuvant is a **detoxified mutant** of a bacterial **ADP-ribosylating toxin**, optionally comprising one or more amino acid additions, deletions or substitutions. The non-toxic mucosal adjuvant may also be a **detoxified mutant** of **cholera toxin** or heat-labile toxin.

IT Toxins
 RL: MOA (Modifier or additive use); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
 (ADP-ribosylating; bacterial ADP-ribosylating or **cholera** or heat-labile **toxins** as nontoxic mucosal adjuvant for antigen vaccine)

IT Bacteria
 Mucous membrane
 Vaccines
 (bacterial ADP-ribosylating or **cholera** or heat-labile **toxins** as nontoxic mucosal adjuvant for antigen vaccine)

IT Antigens
 RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
 (bacterial ADP-ribosylating or **cholera** or heat-labile **toxins** as nontoxic mucosal adjuvant for antigen vaccine)

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09/044696

IT Toxins
RL: MOA (Modifier or additive use); THU (Therapeutic use); BIOL
(Biological study); USES (Uses)
(holo-; bacterial ADP-ribosylating or cholera or
heat-labile toxins as nontoxic mucosal adjuvant for
antigen vaccine)

IT Immunostimulants
(adjuvants, bacterial ADP-ribosylating or cholera or
heat-labile toxins as nontoxic mucosal adjuvant for
antigen vaccine)

IT Toxins
RL: MOA (Modifier or additive use); THU (Therapeutic use); BIOL
(Biological study); USES (Uses)
(cholera, bacterial ADP-ribosylating or cholera or
heat-labile toxins as nontoxic mucosal adjuvant for
antigen vaccine)

IT Toxins
RL: MOA (Modifier or additive use); THU (Therapeutic use); BIOL
(Biological study); USES (Uses)
(heat-labile, bacterial ADP-ribosylating or cholera or
heat-labile toxins as nontoxic mucosal adjuvant for
antigen vaccine)

IT Pharmaceutical dosage forms
(nasal, bacterial ADP-ribosylating or cholera or
heat-labile toxins as nontoxic mucosal adjuvant for
antigen vaccine)

IT Pharmaceutical dosage forms
(oral, bacterial ADP-ribosylating or cholera or
heat-labile toxins as nontoxic mucosal adjuvant for
antigen vaccine)

L3 ANSWER 8 OF 15 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1994:653248 CAPLUS
DOCUMENT NUMBER: 121:253248
TITLE: A genetically detoxified derivative of
heat-labile Escherichia coli enterotoxin induces
neutralizing antibodies against the A subunit
AUTHOR(S): Pizza, Mariagrazia; Fontana, Maria Rita;
Giuliani, Marzia M.; Domenighini, Mario;
Magagnoli, Claudia; Giannelli, Valentina; Nucci,
Daniele; Hol, Wim; Manetti, Roberto; Rappuoli,
Rino
CORPORATE SOURCE: Immunobiological Res. Inst. Siena, Siena, 53100,
Italy
SOURCE: J. Exp. Med. (1994), 180(6), 2147-54
CODEN: JEMEAV; ISSN: 0022-1007
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Escherichia coli enterotoxin (LT) and the homologous
Searcher : Shears 308-4994

cholera toxin (CT) are A-B toxins that cause travelers' diarrhea and cholera, resp. So far, exptl. live and killed vaccines against these diseases have been developed using only the nontoxic B portion of these toxins. The enzymically active A subunit has not been used because it is responsible for the toxicity and it is reported to induce a negligible titer of toxin neutralizing antibodies. Site-directed mutagenesis was used to inactivate the ADP-ribosyltransferase activity of the A subunit. Nontoxic derivs. of LT were obtained that elicited a good titer of neutralizing antibodies recognizing the A subunit.

IT **Escherichia coli**

Protein sequences

(Escherichia coli detoxified enterotoxin deriv. induces neutralizing antibodies against A subunit)

IT **Vaccines**

(for enterotoxigenic Escherichia coli; Escherichia coli detoxified enterotoxin deriv. induces neutralizing antibodies against A subunit)

IT **Toxins**

RL: BAC (Biological activity or effector, except adverse); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(entero-, A subunit; Escherichia coli detoxified enterotoxin deriv. induces neutralizing antibodies against A subunit)

IT **Antibodies**

RL: BPR (Biological process); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative); PROC (Process)
(neutralizing, Escherichia coli detoxified enterotoxin deriv. induces neutralizing antibodies against A subunit)

IT **Mutation**

(site-specific, Escherichia coli detoxified enterotoxin deriv. induces neutralizing antibodies against A subunit)

L3 ANSWER 9 OF 15 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1994:75437 CAPLUS

DOCUMENT NUMBER: 120:75437

TITLE: Genetic detoxification of **pertussis toxin** for vaccine

INVENTOR(S): Klein, Michel H.; Boux, Heather A.; Cockle, Stephen A.; Loosmore, Sheena M.; Zealey, Gavin R.

PATENT ASSIGNEE(S): Connaught Laboratories Ltd., Can.

SOURCE: U.S., 46 pp. Cont-in-part of U.S. 5,085,862.
CODEN: USXXAM

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 2

PATENT INFORMATION:

09/044696

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5244657	A	19930914	US 90-589423	19900928
US 5085862	A	19920204	US 88-275376	19881123
US 5221618	A	19930622	US 91-767837	19910930
US 5332583	A	19940726	US 91-788314	19911105
US 5358868	A	19941025	US 91-788313	19911105
US 5433945	A	19950718	US 92-979798	19921120
PRIORITY APPLN. INFO.:			GB 87-27489	19871124
			US 88-275376	19881123
			US 90-589423	19900928

AB A method is described for the prepn. of a safe, immunogenic, and efficacious vaccine for protection against pertussis. Specific functional sites of **pertussis toxin** have been identified, and, using this information, defined mutant holotoxins have been produced by site-directed mutagenesis of the toxin gene. A no. of these holotoxin analogs are detoxified, retain an immunodominant S1 epitope, are immunogenic, and are protective in the std. pertussis vaccine potency test in mice. The site of interaction of the S1 subunit with NAD was also detd.

IT Plasmid and Episome
(S-3319-3-9 and others, in mutant **pertussis toxin** prodn. vaccine in relation to)

IT Vaccines
(mutant **pertussis toxins** for, **pertussis toxin** detoxification in relation to)

IT Protein sequences
(of **pertussis toxin** mutants)

IT Toxins
RL: BIOL (Biological study)
(pertussis, mutants, for vaccine)

IT 152479-43-1, [.DELTA.9]-**Pertussis toxin** mutant
(Bordetella pertussis 10536 clone S-2679-1-11 S1 subunit)
152479-44-2, [Glu9]-**pertussis toxin** mutant
(Bordetella pertussis 10536 clone S-2815-1-8 S1 subunit)
152479-45-3, [Lys9]-**pertussis toxin** mutant
(Bordetella pertussis 10536 clone S-2953-21 S1 subunit)
152479-46-4, [His9]-**pertussis toxin** mutant
(Bordetella pertussis 10536 clone S-3046-4 S1 subunit)
152479-47-5, [.DELTA.13]-**Pertussis toxin** mutant
(Bordetella pertussis 10536 clone S-2679-2-1 S1 subunit)
152479-48-6, [Glu13]-**pertussis toxin** mutant
(Bordetella pertussis 10536 clone S-2779-2-1 S1 subunit)
152479-49-7, [.DELTA.9-13]-**Pertussis toxin** mutant
(Bordetella pertussis 10536 clone S-2829-2-19 S1 subunit)
152479-50-0, [Glu9Glu13]-**pertussis toxin** mutant
(Bordetella pertussis 10536 clone S-2779-3-2 S1 subunit)
152479-51-1, [Glu58]-**pertussis toxin** mutant

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(Bordetella pertussis 10536 clone J-444-2-2 S1 subunit)
152479-52-2, [.DELTA.57.DELTA.58]-**Pertussis toxin**
mutant (Bordetella pertussis 10536 clone J-482-11 S1 subunit)
152479-53-3, [Ala26]-**pertussis toxin** mutant
(Bordetella pertussis 10536 clone S-3123-2 S1 subunit)
152479-54-4, [Cys26]-**pertussis toxin** mutant
(Bordetella pertussis 10536 clone S-3140-22 S1 subunit)
152479-55-5, [Ala41]-**pertussis toxin** mutant
(Bordetella pertussis 10536 clone S-2515-5-10 S1 subunit)
152479-56-6, [Ser41]-**pertussis toxin** mutant
(Bordetella pertussis 10536 clone S-3124-6 S1 subunit)
152479-57-7, [Ala201]-**pertussis toxin** mutant
(Bordetella pertussis 10536 clone S-2679-3-4 S1 subunit)
152479-58-8, [.DELTA.129]-**Pertussis toxin** mutant
(Bordetella pertussis 10536 clone S-2589-6 S1 subunit) 152479-59-9
, [Gly129]-**pertussis toxin** mutant (Bordetella
pertussis 10536 clone S-2515-3-6 S1 subunit) 152479-60-2,
[Gln129]-**pertussis toxin** mutant (Bordetella
pertussis 10536 clone S-2515-1-2 S1 subunit) 152479-61-3,
[Asp129]-**pertussis toxin** mutant (Bordetella
pertussis 10536 clone S-2515-2-4 S1 subunit) 152479-62-4,
[Asn129]-**pertussis toxin** mutant (Bordetella
pertussis 10536 clone S-2852-1-18 S1 subunit) 152479-63-5,
[Lys129]-**pertussis toxin** mutant (Bordetella
pertussis 10536 clone S-2515-4-11 S1 subunit) 152479-64-6,
[Arg129]-**pertussis toxin** mutant (Bordetella
pertussis 10536 clone M-32-2-4 S1 subunit) 152479-65-7, [His129]-
pertussis toxin mutant (Bordetella pertussis 10536
clone S-2937-1-2 S1 subunit) 152479-66-8, [Pro129]-
pertussis toxin mutant (Bordetella pertussis 10536
clone S-2959-2-28 S1 subunit) 152479-67-9, [Cys129]-
pertussis toxin mutant (Bordetella pertussis 10536
clone J-478-5 S1 subunit) 152479-68-0, [.DELTA.130]-
Pertussis toxin mutant (Bordetella pertussis 10536
clone S-2852-2-1 S1 subunit) 152479-69-1, [Phe130]-
pertussis toxin mutant (Bordetella pertussis 10536
clone S-2836-15 S1 subunit) 152479-70-4, [Gly129Ala130]-
pertussis toxin mutant (Bordetella pertussis 10536
clone S-2679-4-3 S1 subunit) 152479-71-5, [Gln129Ala130]-
pertussis toxin mutant (Bordetella pertussis 10536
clone M-38-1 S1 subunit) 152479-72-6, [Gly129Phe130]-
pertussis toxin mutant (Bordetella pertussis 10536
clone J-444-1-6 S1 subunit) 152479-73-7, [Gln10]-**pertussis**
toxin mutant (Bordetella pertussis 10536 clone S-2995-1-2 S3
subunit) 152479-74-8, [Asn92Arg93]-**pertussis**
toxin mutant (Bordetella pertussis 10536 clone S-2995-2-1 S3
subunit) 152479-75-9, [Asn105]-**pertussis toxin**
mutant (Bordetella pertussis 10536 clone S-2995-3-1 S3 subunit)
152479-76-0, [Ala41Ala201]-**pertussis toxin**

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mutant (Bordetella pertussis 10536 clone S-2818-1 S1 subunit)
 152479-77-1, [Ala41Gly129]-**pertussis toxin**
 mutant (Bordetella pertussis 10536 clone S-2549-2 S1 subunit)
 152479-78-2, [Glu9Gly129]-**pertussis toxin** mutant
 (Bordetella pertussis 10536 clone M-45-1 S1 subunit) 152479-79-3,
 [Glu9Gly129Ala130]-**pertussis toxin** mutant
 (Bordetella pertussis 10536 clone S-2956-1 S1 subunit)
 152479-80-6, [Glu13Gly129]-**pertussis toxin**
 mutant (Bordetella pertussis 10536 clone S-2966-2-13 S1 subunit)
 152479-81-7, [Glu13Gly129Ala130]-**pertussis toxin**
 mutant (Bordetella pertussis 10536 clone S-2961-1 S1 subunit)
 152479-82-8, [.DELTA.9Gln129]-**pertussis toxin**
 mutant (Bordetella pertussis 10536 clone S-2730-1-1 S1 subunit)
 152479-83-9, [.DELTA.9Gln129Ala130]-**pertussis**
toxin mutant (Bordetella pertussis 10536 clone S-2730-3-2 S1
 subunit) 152479-84-0, [.DELTA.13Gln129]-**pertussis**
toxin mutant (Bordetella pertussis 10536 clone S-2730-2-1 S1
 subunit) 152479-85-1, [.DELTA.13Gln129Ala130]-**pertussis**
toxin mutant (Bordetella pertussis 10536 clone S-2730-4-1 S1
 subunit) 152479-86-2, [Lys13]-**pertussis toxin**
 mutant (Bordetella pertussis 10536 clone JB-126-1-1 S1 subunit)
 152479-87-3, [His58]-**pertussis toxin** mutant
 (Bordetella pertussis 10536 clone S-3524-1 S1 subunit)
 152479-88-4, [Lys58]-**pertussis toxin** mutant
 (Bordetella pertussis 10536 clone S-3554-1-1 S1 subunit)
 152479-89-5, [Ala35]-**pertussis toxin** mutant
 (Bordetella pertussis 10536 clone S-3494-1 S1 subunit)
 152479-90-8, [Ser129]-**pertussis toxin** mutant
 (Bordetella pertussis 10536 clone S-3156-1-30 S1 subunit)
 152479-91-9, [Ser130]-**pertussis toxin** mutant
 (Bordetella pertussis 10536 clone S-3502-2-1 S1 subunit)
 152479-92-0, [Glu58Gly129]-**pertussis toxin**
 mutant (Bordetella pertussis 10536 clone S-3305-3 S1 subunit)
 152479-93-1, [Lys9Gly129]-**pertussis toxin** mutant
 (Bordetella pertussis 10536 clone S-3445-3-2 S1 subunit)
 152479-94-2, [Lys9Glu58Gly129]-**pertussis toxin**
 mutant (Bordetella pertussis 10536 clone S-3445-2-14 S1 subunit)
 152479-95-3, [.DELTA.91-93]-**Pertussis toxin**
 mutant (Bordetella pertussis 10536 clone S-3332-1-1 S3 subunit)
 152479-96-4, [.DELTA.91-93]-**Pertussis toxin**
 mutant (Bordetella pertussis 10536 clone S-3290-2-1 S2 subunit)
 RL: BIOL (Biological study)

(amino acid sequence and residual toxicity of, detoxified
pertussis toxin for vaccine in relation to)

IT 103236-62-0, **Pertussis toxin** (Bordetella
 pertussis 10536 clone J-169-1 S2 subunit) 152479-97-5,
Pertussis toxin (Bordetella pertussis 10536 clone
 J-169-1 S1 subunit) 152479-98-6, **Pertussis toxin**
 (Bordetella pertussis 10536 clone J-169-1 S3 subunit)

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RL: BIOL (Biological study)
 (amino acid sequence of and mutants of,
detoxified pertussis toxin for
 vaccine in relation to)

IT 58319-92-9, ADP ribosyltransferase
 RL: BIOL (Biological study)
 (**pertussis toxin** mutants effect on activity
 of)

IT 51-45-6, Histamine, biological studies
 RL: BIOL (Biological study)
 (**pertussis toxin** mutants with decreased
 sensitivity activity of)

IT 53-84-9, NAD
 RL: RCT (Reactant)
 (photocrosslinking of, to **pertussis toxin** S1
 subunit)

L3 ANSWER 10 OF 15 CAPLUS COPYRIGHT 1999 ACS
 ACCESSION NUMBER: 1993:465358 CAPLUS
 DOCUMENT NUMBER: 119:65358
 TITLE: Characterization of **pertussis**
toxin analogs containing mutations in
 B-oligomer subunits

AUTHOR(S): Loosmore, Sheena; Zealey, Gavin; Cockle,
 Stephen; Boux, Heather; Chong, Pele; Yacoob,
 Reza; Klein, Michel

CORPORATE SOURCE: Connaught Cent. Biotechnol. Res., Willowdale,
 ON, M2R 3T4, Can.

SOURCE: Infect. Immun. (1993), 61(6), 2316-24
 CODEN: INFIBR; ISSN: 0019-9567

DOCUMENT TYPE: Journal
 LANGUAGE: English

AB The S2, S3, and S4 subunit genes of **pertussis**
toxin (PT) from Bordetella pertussis were
 subjected to site-directed mutagenesis, and the resultant PT
 analogs assayed for altered biol. properties. PT analogs
 S2(T91,R92,N93).DELTA. and S2(Y102A,Y103A) exhibited reduced binding
 to fetuin. Several PT analogs with mutations in the S2, S3, or S4
 subunit showed reduced in vitro toxicity, as measured in the Chinese
 hamster ovary (CHO) cell clustering assay. In particular, PT
 analogs S3(Y82A) and S3(I91,Y92,K93).DELTA. retained .ltoreq.10%
 residual toxicity. These mutants also exhibited significantly lower
 mitogenic and hemagglutinating activities and reduced in vivo
 activities, as measured by the histamine sensitization and
 leukocytosis assays. The S4(K54A,K57A) PT analog had significantly
 reduced CHO cell clustering activity, though other biol. activities
 remained unaffected. PT analogs S1(E129G)/S3(Y82A) and
 S1(E129G)/S3(I91,Y92,K93).DELTA. displayed a cumulative effect of
 the S1 and S3 mutations for in vitro and in vivo toxic activities.

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These PT analogs, as well as S1(R9K,E129G)/S3(K82A) and S1(R9K,E129G)/S3(I91,Y92,K93).DELTA., still expressed an epitope which elicits a neutralizing antitoxin antibody and were protective in the mouse intracerebral challenge test. Recombinant pertussis vaccines based on PT analogs with **detoxifying mutations** in multiple subunits may thus represent the next generation of improved whooping cough vaccines.

IT Mutation

(in B-oligomer subunits of **pertussis toxin** analogs, characterization of)

IT Toxins

RL: BIOL (Biological study)

(pertussis, analogs, mutations in B-oligomer subunits, characterization of)

L3 ANSWER 11 OF 15 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1992:544511 CAPLUS

DOCUMENT NUMBER: 117:144511

TITLE: Construction of Bordetella pertussis strains that overproduce genetically inactivated **pertussis toxin**

AUTHOR(S): Zealey, Gavin; Loosmore, Sheena; Yacoob, Reza; Cockle, Stephen; Herbert, Andy; Klein, Michel

CORPORATE SOURCE: Res. Connaught Lab., Connaught Cent. Biotechnol., Willowdale, ON, M2R 3T4, Can.

SOURCE: Vaccines 92: Mod. Approaches New Vaccines Incl. Prev. AIDS [Annu. Meet.], 9th (1992), 367-71. Editor(s): Brown, Fred. Cold Spring Harbor Lab. Press: Cold Spring Harbor, N. Y. CODEN: 57WXAL

DOCUMENT TYPE: Conference

LANGUAGE: English

AB **Pertussis toxin (PT)** is a major

protective antigen in pertussis vaccines. For max. immunogenicity and safety, PT should be detoxified by genetic rather than chem. means. Such detoxification has been achieved by site-directed mutagenesis of the tox operon and prodn. of nontoxic PT analogs by recombinant Bordetella pertussis strains (M. A. Pizza et al. 1989, S. M. Loosmore et al. 1990). One highly **detoxified** PT analog contains the **mutations** Arg-9.fwdarw.Lys and Glu-129.fwdarw.Gly in subunit S1. This mol. is immunogenic and protective and is an appropriate antigen for inclusion in an acellular whooping cough vaccine. However, the relatively low level of PT secretion by B. pertussis is a limiting factor in the prodn. of such analogs. To increase the secretion of the Lys9Gly129 PT analog by B. pertussis, addnl. copies of the mutated tox operon were integrated at the tox and fha loci by unmarked allelic exchange. The resulting recombinant strains secrete amts. of PT analog proportional to gene dosage, and yields of up to 80 mg/L can be

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obtained in 10-L fermentors.

IT Toxins

RL: BIOL (Biological study)

(pertussis, overprodn. of inactivated, by recombinant Bordetella pertussis)

L3 ANSWER 12 OF 15 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1991:406584 CAPLUS

DOCUMENT NUMBER: 115:6584

TITLE: Detoxification of **pertussis**

toxin by site-directed mutagenesis: a

review of Connaught strategy to develop a recombinant pertussis vaccine

AUTHOR(S): Loosmore, Sheena; Cockle, Stephen; Zealey, Gavin; Boux, Heather; Phillips, Kimberley; Fahim, Raafat; Klein, Michel

CORPORATE SOURCE: Connaught Cent. Biotechnol., Willowdale, ON, M2R 3T4, Can.

SOURCE: Mol. Immunol. (1991), 28(3), 235-8

CODEN: MOIMD5; ISSN: 0161-5890

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The authors identified and mutated several crit. amino acid residues in subunit S1 of **pertussis toxin** to generate highly detoxified PT analogs. Several of these analogs were scaled-up, purified, tested as potential vaccine candidates in exptl. animals, and found to be both immunogenic and protective. These **pertussis toxin** analogs, or derivs. thereof, will serve to design the new generation of recombinant acellular pertussis vaccines.

IT Vaccines

(for **pertussis**, detoxification of **pertussis**

toxin by site-directed mutagenesis in development of)

IT Detoxication

(of **pertussis toxin**, by site-directed mutagenesis, in vaccine development)

IT Bordetella **pertussis**

(vaccine against, **toxin** detoxification by site-directed **mutagenesis** in development of)

IT Mutation

(site-specific, of **pertussis toxin**, in vaccine development)

IT 58319-92-9, ADP-Ribosyltransferase

RL: BIOL (Biological study)

(of **pertussis toxin** analogs, in vaccine development)

IT 51-45-6, Histamine, biological studies

RL: BIOL (Biological study)

(sensitization by, **pertussis toxin** analogs

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induction of, in vaccine development)

L3 ANSWER 13 OF 15 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1991:241885 CAPLUS

DOCUMENT NUMBER: 114:241885

TITLE: Gene replacement in Bordetella pertussis by transformation with linear DNA

AUTHOR(S): Zealey, G. R.; Loosmore, S. M.; Yacoob, R. K.; Cockle, S. A.; Boux, L. J.; Miller, L. D.; Klein, M. H.

CORPORATE SOURCE: Connaught Cent. Biotechnol. Res., Willowdale, ON, M2R 3T4, Can.

SOURCE: Bio/Technology (1990), 8(11), 1025-9
CODEN: BTCHDA; ISSN: 0733-222X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The wild-type TOX operon of B. pertussis was replaced with in vitro mutated, detoxified alleles by electroporetic transformation using unmarked linear DNA. Uptake of DNA was selected by transient ampicillin resistance. Two simultaneous recombination events resulted in gene-replacement at the natural locus without integration of heterologous DNA. TOX Alleles were stable without selection and recombinant strains secreted non-toxic, fully assembled, protective pertussis toxin (PT) analogs with kinetics similar to the parental vaccine strain under prodn.-scale fermn. conditions. Strains generated in this way are suitable for the prodn. of recombinant whole-cell or component whooping cough vaccines that require no chem. modification of PT.

IT Transformation, genetic
(electroporation-mediated, of Bordetella pertussis, pertussis toxin gene replacement by, vaccine development in relation to)

IT Gene and Genetic element, microbial
RL: BIOL (Biological study)
(for pertussis toxin, of Bordetella pertussis, electroporation-mediated substitution of detoxified alleles for, vaccine development in relation to)

IT Vaccines
(non-toxic protective pertussis toxin analogs for, gene replacement in Bordetella pertussis in relation to)

IT Fermentation
(of recombinant Bordetella pertussis, for prodn. of recombinant non-toxic pertussis toxin analogs, for vaccine prodn.)

IT Bordetella pertussis
(TOX operon of, electroporation-mediated substitution of, detoxified alleles in, vaccine prodn. in relation to)

IT Toxins

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RL: BIOL (Biological study)
 (pertussis, operon for, of Bordetella pertussis,
 electroporation-mediated replacement of, vaccine development in
 relation to)

IT Operon
 (tox, for **pertussis toxin**, of Bordetella
 pertussis, electroporetic transformation for replacement of,
 vaccine development in relation to)

L3 ANSWER 14 OF 15 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1990:629139 CAPLUS

DOCUMENT NUMBER: 113:229139

TITLE: Engineering of genetically detoxified
pertussis toxin analogs for
 development of a recombinant whooping cough
 vaccine

AUTHOR(S): Loosmore, Sheena M.; Zealey, Gavin R.; Boux,
 Heather A.; Cockle, Stephen A.; Radika, Kesavan;
 Fahim, Raafat E. F.; Zobrist, Gloria J.; Yacoob,
 Reza K.; Chong, Pele C. S.; et al.

CORPORATE SOURCE: Connaught Cent. Biotechnol. Res., Willowdale,
 ON, M2R 3T4, Can.

SOURCE: Infect. Immun. (1990), 58(11), 3653-62
 CODEN: INFIBR; ISSN: 0019-9567

DOCUMENT TYPE: Journal

LANGUAGE: English

AB **Pertussis toxin (PT)** is an important
 protective antigen in vaccines against whooping cough, and a
 genetically detoxified **PT** analog is the preferred form of
 the immunogen. Several amino acids of the S1 subunit were
 identified as functionally crit. residues by site-directed
 mutagenesis, specifically, those at positions 9, 13, 26, 35, 41, 58,
 and 129. Eighty-three mutated **PT** operons were introduced
 into Bordetella parapertussis, and the resultant **toxin**
 analogs were screened for expression levels, enzymic activity,
 residual toxicity, and antigenicity. While more than half of the
 mutants were poorly secreted or assembled, the rest were fully
 assembled and most were highly detoxified. Single mutations
 resulted in up to a 1,000-fold redn. in both toxic and enzymic
 activities, while **PT** analogs with multiple mutations (Lys-9 Gly-129,
 Glu-58 Gly-129, and Lys-9 Glu-58 Gly-129) were 106-fold detoxified.
 Operons coding for stable and nontoxic mutants shown to express a
 crit. immunodominant protective epitope were returned to the
 chromosome of B. pertussis by allelic exchange. In vivo anal. of
 the **toxin** analogs showed a dramatic redn. in histamine sensitization
 and lymphocytosis-promoting activities, paralleling the redn. in
 toxic activities. All mutants were protective in an intracerebral
 challenge test, and the Lys-9 Gly-129 analog was more immunogenic
 than the toxoid. **PT** analogs such as those described represent

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suitable components for the design of a recombinant whooping cough vaccine.

- IT Vaccines
(against whooping cough, recombinant detoxified **pertussis toxin** analogs as)
- IT Mutation
(in **detoxified** pertussis analogs prepn.)
- IT Detoxication
(of **pertussis toxin** recombinant analogs, in vaccine prepn.)
- IT Whooping cough
(vaccine against, recombinant detoxified **pertussis toxin** analogs as)
- IT Toxins
RL: BIOL (Biological study)
(pertussis, recombinant analogs of, as vaccine against whooping cough)

L3 ANSWER 15 OF 15 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1990:404262 CAPLUS

DOCUMENT NUMBER: 113:4262

TITLE: Detoxification of **pertussis toxin** by site-directed mutagenesis

AUTHOR(S): Cockle, S.; Loosmore, S.; Radika, K.; Zealey, G.; Boux, H.; Phillips, K.; Klein, M.

CORPORATE SOURCE: Connaught Res. Inst., Willowdale, ON, M2R 3T4, Can.

SOURCE: Adv. Exp. Med. Biol. (1989), 251(Immunobiol. Proteins Pept. 5), 209-14
CODEN: AEMBAP; ISSN: 0065-2598

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A series of pertussis holotoxin (PT) analogs was generated in engineered Bordetella strains by mutation of the catalytic S1 subunit at 7 crit. sites, including Glu129, which was found by photocross-linking to be near the active site for NAD hydrolysis. Many of these **mutants** were highly **detoxified** according to CHO cell clustering and ADPR assays, and retained an immunodominant protective S1 epitope. Three affinity-purified analogs were immunogenic and protective in mice, yet exhibited low toxicity. This is an important advance towards the development of a genetically inactivated form of PT for inclusion in a new generation of whooping cough vaccines.

- IT Vaccines
(for **pertussis, pertussis toxin** detoxification by site-directed mutagenesis in relation to)
- IT Detoxication
(of **pertussis toxin**, by site-directed

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mutagenesis, vaccine in relation to)

IT Toxins

RL: BIOL (Biological study)

(pertussis, site-directed mutations in, vaccine toxicity in relation to)

IT Mutation

(site-specific, of **pertussis toxin**, vaccine in relation to)

=> d his 14-; d 1-19 ibib abs

(FILE 'MEDLINE, BIOSIS, EMBASE, TOXLIT, TOXLINE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, CABA, AGRICOLA' ENTERED AT 15:21:16 ON 27 JUL 1999)

L4 37 S L3

L5 19 DUP REM L4 (18 DUPLICATES REMOVED)

L5 ANSWER 1 OF 19 TOXLIT

ACCESSION NUMBER: 1998:68701 TOXLIT

DOCUMENT NUMBER: CA-128-279567V

TITLE: Immunogenic **detoxified mutant**
Escherichia coli LT-A
toxin.

AUTHOR: Pizza M; Giuliani MM; Rappuoli R

SOURCE: (1998). PCT Int. Appl. PATENT NO. 9818928 05/07/1998
(Rappuoli, Rino).
CODEN: PIXXD2.

PUB. COUNTRY: ITALY

DOCUMENT TYPE: Patent

FILE SEGMENT: CA

LANGUAGE: English

OTHER SOURCE: CA 128:279567

ENTRY MONTH: 199806

AB An immunogenic detoxified protein is provided which comprises the amino acid sequence of subunit A of an *E. coli* heat labile **toxin (LT-A)** or a fragment thereof in which at least amino acid Ala-72 of the A subunit is mutated, preferably by substitution with Arg. The toxoid is useful as vaccine against an enterotoxigenic strain of *E. coli* and is produced by recombinant DNA means by site-directed mutagenesis. A 1.5 kb *Sma*I-*Eco*RI fragment from plasmid pEWD299 contg. the gene for **LT-A** and the **LT** promoter region was subcloned to produce vector **BS-LT-A**. **BS-LT-A** was mutagenized with oligonucleotide oligo**LT-A72R** to change the Ala-72 codon to the Arg codon and ligated to the *Eco*RI-*Hind*III fragment contg. the gene for **LT-B** and cloned to produce vector **BS-LTA72R**. *E. coli* was transformed with **BS-LTA72R** and the **LT-A72R** mutant purified. ADP-ribosylation of **LT-A72R** was lower than wild type **LT-A**, the toxicity of **LT-A72R** was 10⁻⁵ lower than

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wild type **LT-A**, and **LT-A72R** proved to be an effective mucosal adjuvant.

L5 ANSWER 2 OF 19 WPIDS COPYRIGHT 1999 DERWENT INFORMATION LTD

ACCESSION NUMBER: 1999-070064 [06] WPIDS

DOC. NO. CPI: C99-020598

TITLE: **Detoxified mutants** of bacterial

ADP-ribosylating toxins

as parenteral adjuvants - useful to enhance humoral and cell-mediated immune responses in vertebrates when administered with selected antigen e.g. in disease treatment.

DERWENT CLASS: B04 D16

INVENTOR(S): BARCHFELD, G; DEL GIUDICE, G; RAPPUOLI, R

PATENT ASSIGNEE(S): (CHIR) CHIRON CORP

COUNTRY COUNT: 80

PATENT INFORMATION:

PATENT NO KIND DATE WEEK LA PG

WO 9842375 A1 981001 (9906)* EN 51

RW: AT BE CH DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW
NL OA PT SD SE SZ UG ZW

W: AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI
GB GE GH GM GW HU ID IL IS JP KE KG KP KR KZ LC LK LR LS LT
LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL
TJ TM TR TT UA UG UZ VN YU ZW

AU 9865713 A 981020 (9909)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9842375	A1	WO 98-US5454	980319
AU 9865713	A	AU 98-65713	980319

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 9865713	A Based on	WO 9842375

PRIORITY APPLN. INFO: US 98-44696 980318; US 97-41227 970321

AN 1999-070064 [06] WPIDS

AB WO 9842375 A UPAB: 19990210

A parenteral adjuvant composition is new, comprising a **detoxified mutant** of a bacterial **ADP-ribosylating toxin** as the parenteral adjuvant, at least one selected antigen and optionally a pharmaceutically

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acceptable (optionally topical) vehicle. In the disclosure 'Detoxified' mutants are defined as completely non-toxic/having low residual toxicity (preferably less than 0.01 % of natural counterparts) as measured by e.g. morphological changes induced in Y1 cells.

USE - The adjuvant composition can be administered parenterally in conjunction with at least one antigen in methods to immunise vertebrate subjects (claimed). The adjuvant has the ability to enhance the humoral and cell-mediated immune responses elicited by the antigen (e.g. by making the antigen more strongly immunogenic or necessitating fewer/lower antigen doses). It can be administered prior/subsequent to the antigen, and is preferably administered within a short space of time to the same site; it can also be administered in isolation from antigens as a boost following systemic or mucosal antigen administration.

Most preferably, the adjuvant is co-administered with the antigen in the compositions and a pharmaceutically acceptable carrier. The antigen may be derived from viruses, bacteria, parasites and fungi or may be tumour antigens, self-antigens and allergens. The compositions are therefore useful in the treatment and prevention of e.g. viral diseases, allergic manifestations, diseases caused by pathogens (e.g. bacteria or parasites), AIDS, autoimmune diseases (e.g. Systemic Lupus Erythematosus), Alzheimer's disease and cancers. The adjuvant can also be used to prepare antibodies against selected antigen(s), useful e.g. for diagnostic purposes or for antigen purification. The composition of can also be used to manufacture medicaments useful for parenterally immunising (e.g. subcutaneously, intramuscularly or especially transcutaneously) vertebrates (claimed).

ADVANTAGE - The adjuvant compositions function when administered parenterally, so allow immunity to be conferred to substances not amenable to other modes of administration (e.g. oral or intranasal delivery).

Dwg.0/2

L5	ANSWER 3 OF 19	MEDLINE	DUPLICATE 1
ACCESSION NUMBER:	1998347282	MEDLINE	
DOCUMENT NUMBER:	98347282		
TITLE:	Mucosal immunogenicity of genetically detoxified derivatives of heat labile toxin from Escherichia coli.		
AUTHOR:	Douce G; Giuliani M M; Giannelli V; Pizza M G; Rappuoli R; Dougan G		
CORPORATE SOURCE:	Department of Biochemistry, Imperial College of Science, Technology and Medicine, London, UK.		
SOURCE:	VACCINE, (1998 Jul) 16 (11-12) 1065-73.		
	Journal code: X60. ISSN: 0264-410X.		
PUB. COUNTRY:	ENGLAND: United Kingdom		
	Journal; Article; (JOURNAL ARTICLE)		
	Searcher : Shears 308-4994		

09/044696

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199812
ENTRY WEEK: 19981204

AB Using a fixed dose of antigen, the immune response to **detoxified mutants** of LT-WT following intranasal (i.n.), subcutaneous (s.c.) and oral (i.g.) immunisation has been studied. When given i.n., both LT-WT and mutant **toxin**, K63, generated significant levels of **toxin**-specific IgG in the serum, and the levels of IgA in nasal and lung lavages were greater than those induced by rLT-B. In comparison, i.g. immunisation of mice with a similar quantity of either LT-WT or K63 **toxin** induced barely detectable levels of IgG in the sera. However, if the amount of protein used for i.g. immunisation was increased tenfold, relatively good levels of **toxin**-specific IgG were induced in the sera by both LT-WT or K63. Low levels of **toxin**-specific IgA were also observed in intestinal washes from these mice. Western blotting of the sera, using the native **toxin** as an antigen, demonstrated the presence of both anti-A and anti-B subunit antibodies. Most significantly, **toxin**-neutralising antibodies were induced in the serum, with the strongest activity being induced by the LT-WT, an intermediate activity induced by mutant K63 and a lower response by rLT-B. Together, these data show that ADP-ribosyltransferase is not necessary for mucosal immunogenicity of these proteins, and that the i.n. route of immunisation is more effective than the i.g. route of immunisation for the generation of both systemic (IgG) and mucosal (IgA) immune responses.

L5 ANSWER 4 OF 19 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 1998307520 MEDLINE
DOCUMENT NUMBER: 98307520
TITLE: **Pertussis toxin** potentiates Th1 and Th2 responses to co-injected antigen: adjuvant action is associated with enhanced regulatory cytokine production and expression of the co-stimulatory molecules B7-1, B7-2 and CD28.
AUTHOR: Ryan M; McCarthy L; Rappuoli R; Mahon B P; Mills K H
CORPORATE SOURCE: Department of Biology, National University of Ireland, Maynooth, Co. Kildare.
SOURCE: INTERNATIONAL IMMUNOLOGY, (1998 May) 10 (5) 651-62.
Journal code: AY5. ISSN: 0953-8178.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199811
ENTRY WEEK: 19981102

Searcher : Shears 308-4994

AB Pertussis toxin (PT) is a major virulence factor of *Bordetella pertussis* which exerts a range of effects on the immune system, including the enhancement of IgE, IgA and IgG production, delayed-type hypersensitivity reactions, and the induction of experimental autoimmune diseases. However, the mechanism by which PT mediates adjuvanticity remains to be defined. In this investigation we have shown that PT can potentiate antigen-specific T cell proliferation and the secretion of IFN-gamma, IL-2, IL-4 and IL-5 when injected with foreign antigens. A chemically detoxified PT and a genetic mutant with substitutions/deletions in the S-1 and B oligomer components that abrogate enzymatic and binding activity displayed no adjuvant properties. In contrast, a non-toxic S-1 mutant devoid of enzymatic activity but still capable of receptor binding retained its adjuvanticity, augmenting the activation of both Th1 and Th2 subpopulations of T cells. In an attempt to address the mechanism of T cell activation, we found that PT stimulated the production of IFN-gamma and IL-2 by naive T cells and IL-1 by macrophages. Therefore potentiation of distinct T cell subpopulations may have resulted in part from the positive influence of IFN-gamma on the development of Th1 cells and the co-stimulatory role of IL-1 for Th2 cells. Furthermore, PT augmented expression of the co-stimulatory molecules B7-1 and B7-2 on macrophages and B cells, and CD28 on T cells, suggesting that the adjuvant effect may also be associated with facilitation of the second signal required for maximal T cell activation. This study demonstrates that the immunopotentiating properties of PT are largely independent of ADP-ribosyltransferase activity, but are dependent on receptor binding activity and appear to involve enhanced activation of T cells.

L5 ANSWER 5 OF 19 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.
 ACCESSION NUMBER: 1998074807 EMBASE
 TITLE: Recent advances in immunological adjuvants: The development of particulate antigen delivery systems.
 AUTHOR: O'Hagan D.T.
 CORPORATE SOURCE: D.T. O'Hagan, Chiron Corporation, 4560 Horton Street, Emeryville, CA 94704, United States
 SOURCE: Expert Opinion on Investigational Drugs, (1998) 7/3 (349-359).
 Refs: 70
 ISSN: 1354-3784 CODEN: EOIDER
 COUNTRY: United Kingdom
 DOCUMENT TYPE: Journal; General Review
 FILE SEGMENT: 004 Microbiology
 026 Immunology, Serology and Transplantation
 030 Pharmacology
 037 Drug Literature Index
 039 Pharmacy
 Searcher : Shears 308-4994

LANGUAGE: English

SUMMARY LANGUAGE: English

AB New generation vaccines, including those based on recombinant proteins, are safer than traditional vaccines, but are less immunogenic. Therefore, there is an urgent need for the development of new and improved vaccine adjuvants. A number of potent immunostimulatory molecules obtained from bacterial cells or plants have been extensively evaluated as adjuvants. However, a number of these molecules have displayed significant toxicity, both in preclinical animal models and in human clinical trials. An alternative approach to the development of novel adjuvants involves the preparation of particulate antigen delivery systems of similar dimensions to natural pathogens. In the absence of additional immunostimulatory molecules, emulsion droplets and microparticles have been shown to be potent adjuvants for the induction of both humoral and cell-mediated immune responses following systemic administration. Moreover, particulate delivery systems have been shown to display an acceptable toxicity profile in a number of clinical trials. Particulate antigen delivery systems also have the potential to function as potent adjuvants following administration by mucosal routes, including oral and intranasal. An alternative approach to the mucosal delivery of vaccines involves the use of genetically **detoxified mutant toxins**, e.g., **LT-K63**, as mucosal adjuvants. The use of novel adjuvants and antigen delivery systems is likely to extend the use of vaccines into the area of therapeutics, involving the eradication of infectious diseases and cancers, or the amelioration of autoimmune disorders.

L5 ANSWER 6 OF 19 EMBASE COPYRIGHT 1999 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 1998243582 EMBASE

TITLE: Eradication of chronic *Helicobacter pylori* infection by therapeutic vaccination.

AUTHOR: Crabtree J.E.

CORPORATE SOURCE: J.E. Crabtree, Molecular Medicine Unit, Clinical Sciences Building, St James's University Hospital, Leeds LS9 7TF, United Kingdom

SOURCE: Gut, (1998) 43/1 (7-8).

Refs: 24

ISSN: 0017-5749 CODEN: GUTTAK

COUNTRY: United Kingdom

DOCUMENT TYPE: Journal; (Short Survey)

FILE SEGMENT: 004 Microbiology
026 Immunology, Serology and Transplantation
030 Pharmacology
037 Drug Literature Index
048 Gastroenterology

LANGUAGE: English

SUMMARY LANGUAGE: English

Searcher : Shears 308-4994

AB Chronic infection of the gastroduodenal mucosae by the gram-negative spiral bacterium *Helicobacter pylori* is responsible for chronic active gastritis, peptic ulcers, and gastric cancers such as adenocarcinoma and low- grade B-cell lymphoma. The success of eradication by antibiotic therapy is being rapidly hampered by the increasing occurrence of antibiotic-resistant strains. An attractive alternative approach to combat this infection is represented by the therapeutic use of vaccines. In the present work, we have exploited the mouse model of persistent infection by mouse-adapted *H. pylori* strains that we have developed to assess the feasibility of the therapeutic use of vaccines against infection. We report that an otherwise chronic *H. pylori* infection in mice can be successfully eradicated by intragastric vaccination with *H. pylori* antigens such as recombinant VacA and CagA, which were administered together with a genetically detoxified mutant of the heat-labile enterotoxin of *Escherichia coli* (referred to as LTK63), in which the serine in position 63 was replaced by a lysine. Moreover, we show that therapeutic vaccination confers efficacious protection against reinfection. These results represent strong evidence of the feasibility of therapeutic use of VacA- or CagA-based vaccine formulations against *H. pylori* infection in an animal model and give substantial preclinical support to the application of this kind of approach in human clinical trials.

L5 ANSWER 7 OF 19 WPIDS COPYRIGHT 1999 DERWENT INFORMATION LTD
 ACCESSION NUMBER: 1997-424757 [39] WPIDS
 DOC. NO. CPI: C97-135893
 TITLE: Immunogenic detoxified mutants
 of cholera toxin - produced by
 substitution of at least one amino acid, gives a
 toxin with lower toxicity than natural toxin, used
 in cholera vaccine.
 DERWENT CLASS: B04 D16
 INVENTOR(S): FONTANA, M R; PIZZA, M; RAPPUOLI, R
 PATENT ASSIGNEE(S): (CHIR-N) CHIRON SPA
 COUNTRY COUNT: 21
 PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9729771	A1	970821	(9739)*	EN	54
RW: AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE					
W: CA JP US					
EP 880361	A1	981202	(9901)	EN	
R: AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE					

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
		Searcher : Shears	308-4994

09/044696

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WO 9729771  A1          WO 97-IB183    970217  
EP 880361   A1          EP 97-902552   970217  
WO 97-IB183    970217
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FILING DETAILS:

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PATENT NO  KIND          PATENT NO  
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EP 880361   A1 Based on  WO 9729771
```

PRIORITY APPLN. INFO: GB 96-3314 960216

AN 1997-424757 [39] WPIDS

AB WO 9729771 A UPAB: 19970926

A novel immunogenic detoxified protein (I) comprises the amino acid sequence of subunit A of a cholera toxin (CT-A) or its fragment, in which at least amino acid is substituted by another amino acid. Purified (I) has a residual toxicity which is greater than 10000 times lower than the toxicity of its natural counterpart.

USE - (I) is used as a vaccine against Vibrio cholerae in mammals (claimed).

ADVANTAGE - The vaccine provides longer lasting protection than the conventional vaccine of killed bacteria, without special side effects.

Dwg.0/0

L5 ANSWER 8 OF 19 WPIDS COPYRIGHT 1999 DERWENT INFORMATION LTD

ACCESSION NUMBER: 1997-108961 [10] WPIDS

DOC. NO. CPI: C97-034829

TITLE: New immunogenic detoxified proteins - comprising cholera toxin A or E. coli heat labile toxin A sub-unit with substitutions at Ser-63 and Arg-192..

DERWENT CLASS: B04 D16

INVENTOR(S): FONTANA, M R; GIANNELLI, V; PIZZA, M; RAPPUOLI, R

PATENT ASSIGNEE(S): (BIOC-N) BIOCINE SPA; (CHIR) CHIRON SPA

COUNTRY COUNT: 22

PATENT INFORMATION:

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PATENT NO  KIND  DATE    WEEK    LA    PG  
-----  
WO 9702348  A1  970123 (9710)* EN    64  
RW: AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE  
W: AU CA JP US  
AU 9662388  A   970205 (9721)  
EP 835314   A1  980415 (9819) EN  
R: AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE
```

Searcher : Shears 308-4994

09/044696

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9702348	A1	WO 96-IB703	960701
AU 9662388	A	AU 96-62388	960701
EP 835314	A1	EP 96-921043	960701
		WO 96-IB703	960701

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 9662388	A Based on	WO 9702348
EP 835314	A1 Based on	WO 9702348

PRIORITY APPLN. INFO: GB 95-13371 950630

AN 1997-108961 [10] WPIDS

AB WO 9702348 A UPAB: 19990316

An immunogenic detoxified protein (IDP) is claimed, comprising the amino acid sequence of subunit A of a **cholera toxin (CT-A)** or a fragment of the amino acid sequence of subunit A of an **E. coli** heat labile **toxin (LT-A)** or a fragment where the amino acids at, or in positions corresponding to, Ser-63 and Arg-192 are replaced with another amino acid. Also claimed are: (1) a DNA sequence encoding an IDP as above; (2) a vector carrying a DNA as in (1); and (3) a host cell line transformed with a vector as in (2).

USE - The IDPs can be administered for the prevention or treatment of a disease caused by *Vibrio cholerae* or an enterotoxigenic strain of *E. coli*. They can also be used as mucosal adjuvants for other immunogenic proteins

ADVANTAGE - The **mutation** at Ser-63 **detoxifies** the toxins while the **mutation** at Arg-192 markedly improves the stability of the resulting protein.

Dwg.0/7

L5 ANSWER 9 OF 19 BIOSIS COPYRIGHT 1999 BIOSIS

ACCESSION NUMBER: 1994:325732 BIOSIS

DOCUMENT NUMBER: PREV199497338732

TITLE: Characterization of PT analogs with
detoxifying mutations in the B
subunit genes.

AUTHOR(S): Loosmore, S.; Zealey, G.; Cockle, S.; Boux, H.;
Yacoob, R.; Klein, M.

CORPORATE SOURCE: Connaught Cent. Biotechnol. Res., 1755 Steeles Ave.
W., Willowdale, ON M2R 3T4 Canada

SOURCE: Freer, J. [Editor]; Aitken, R. [Editor]; Alouf, J. E.
[Editor]; Boulnois, G. [Editor]. FEMS Symposium,
Searcher : Shears 308-4994

09/044696

(1994) No. 73, pp. 408-409. FEMS Symposium; Bacterial protein toxins.

Publisher: Gustav Fischer Verlag Wollgrasweg 49, D-7000 Stuttgart, Germany.

Meeting Info.: Sixth European Workshop Stirling, Scotland, UK June 27-July 2, 1993

ISSN: 0163-9188. ISBN: 3-437-11535-9, 1-56081-385-7.

DOCUMENT TYPE: Book; Conference

LANGUAGE: English

L5 ANSWER 10 OF 19 WPIDS COPYRIGHT 1999 DERWENT INFORMATION LTD

ACCESSION NUMBER: 1993-227320 [28] WPIDS

DOC. NO. CPI: C93-101279

TITLE: Immunogenic detoxified mutant cholera toxin and heat labile toxin - useful as vaccines against infection by Vibrio cholerae and enterotoxin producing Escherichia coli.

DERWENT CLASS: B04 D16

INVENTOR(S): DOMENIGHINI, M; HOL, W; PIZZA, M; RAPPUOLI, R

PATENT ASSIGNEE(S): (BIOC-N) BIOCINE SCLAVO SPA; (BIOC-N) BIOCINE SPA; (CHIR) CHIRON SPA; (CHIR-N) CHIRON SPA; (ISTS) SCLAVO RICERCA SRL

COUNTRY COUNT: 42

PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG

WO 9313202	A1	930708	(9328)*	EN	60
RW: AT BE CH DE DK ES FR GB GR IE IT LU MC NL OA PT SE					
W: AT AU BB BG BR CA CH CS DE DK ES FI GB HU JP KP KR LK LU MG					
MN MW NL NO NZ PL PT RO RU SD SE US					
AU 9333476	A	930728	(9347)		
EP 620850	A1	941026	(9441)	EN	
R: AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE					
TW 239146	A	950121	(9515)		
JP 07506240	W	950713	(9536)		20
IT 1253009	B	950710	(9608)		
SG 48217	A1	980417	(9828)		
EP 869181	A1	981007	(9844)	EN	
R: AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE					
EP 620850	B1	990303	(9913)	EN	
R: AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE					
DE 69228563	E	990408	(9920)		
ES 2127808	T3	990501	(9924)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
		Searcher : Shears	308-4994

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WO 9313202	A1	WO 92-EP3016	921230
AU 9333476	A	AU 93-33476	921230
EP 620850	A1	WO 92-EP3016	921230
		EP 93-902138	921230
TW 239146	A	TW 93-100298	930118
JP 07506240	W	WO 92-EP3016	921230
		JP 93-511447	921230
IT 1253009	B	IT 91-MI3513	911231
SG 48217	A1	SG 96-8033	921230
EP 869181	A1 Div ex	EP 93-902138	921230
		EP 98-200534	921230
EP 620850	B1	WO 92-EP3016	921230
		EP 93-902138	921230
	Related to	EP 98-200534	921230
DE 69228563	E	DE 92-628563	921230
		WO 92-EP3016	921230
		EP 93-902138	921230
ES 2127808	T3	EP 93-902138	921230

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 9333476	A Based on	WO 9313202
EP 620850	A1 Based on	WO 9313202
JP 07506240	W Based on	WO 9313202
EP 869181	A1 Div ex	EP 620850
EP 620850	B1 Related to	EP 869181
	Based on	WO 9313202
DE 69228563	E Based on	EP 620850
	Based on	WO 9313202
ES 2127808	T3 Based on	EP 620850

PRIORITY APPLN. INFO: IT 91-MI3513 911231

AN 1993-227320 [28] WPIDS

AB WO 9313202 A UPAB: 19990316

An immunogenic detoxified protein (I) comprises (a) the amino acid sequence of subunit (A) of a cholera toxin (CT-A) (fragment) or (b) the amino acid sequence of subunit A of an Escherichia coli heat labile toxin (LT-A) (fragment); where at least 1 amino acid(s) at or in positions Val-53, Ser-63, Val-97, Tyr-104, or Pro-106 are replaced with another amino acid.

Also new are: (1) an immunogenic compsn. for use as a vaccine comprising (I) and a pharmaceutically acceptable carrier; (2) the DNA sequence encoding (I); (3) a vector carrying the sequence of (2); (4) a host cell line transformed with the vector of (3); (5) prodn. of (I) comprising culturing the cell (4); (6) prodn. of the

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DNA of (2) by subjecting a DNA encoding a CT-A or an LT-A (fragment) to site-directed mutagenesis; (7) the use of the vaccine of (1) to vaccinate a mammal against *Vibrio cholerae* or an enterotoxigenic strain of *E. coli*; and (8) a process for the formulation of the vaccine of (7).

USE/ADVANTAGE - (I) can be used to give total protection against cholera or enterotoxigenic *E. coli*. It retains immunogenic properties but has significantly reduced or absent toxicity
Dwg.0/3

L5 ANSWER 11 OF 19 MEDLINE

DUPLICATE 3

ACCESSION NUMBER: 93273477 MEDLINE

DOCUMENT NUMBER: 93273477

TITLE: Characterization of **pertussis toxin**
analogs containing mutations in B-oligomer subunits.AUTHOR: Loosmore S; Zealey G; Cockle S; Boux H; Chong P;
Yacoob R; Klein MCORPORATE SOURCE: Connaught Centre for Biotechnology Research,
Willowdale, Ontario, Canada..SOURCE: INFECTION AND IMMUNITY, (1993 Jun) 61 (6) 2316-24.
Journal code: G07. ISSN: 0019-9567.PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals; Cancer Journals

ENTRY MONTH: 199309

AB The S2, S3, and S4 subunit genes of **pertussis toxin (PT)** from *Bordetella pertussis* were subjected to site-directed mutagenesis, and the resultant PT analogs were assayed for altered biological properties. PT analogs S2(T91,R92,N93) delta and S2(Y102A,Y103A) exhibited reduced binding to fetuin. Several PT analogs with mutations in the S2, S3, or S4 subunit showed reduced in vitro toxicity, as measured in the Chinese hamster ovary (CHO) cell clustering assay. In particular, PT analogs S3(Y82A) and S3(I91,Y92,K93) delta retained 10% or less residual toxicity. These mutants also exhibited significantly lower mitogenic and hemagglutinating activities and reduced in vivo activities, as measured by the histamine sensitization and leukocytosis assays. The S4(K54A,K57A) PT analog had significantly reduced CHO cell clustering activity, though other biological activities remained unaffected. PT analogs S1(E129G)/S3(Y82A) and S1(E129G)/S3(I91,Y92,K93) delta displayed a cumulative effect of the S1 and S3 mutations for both in vitro and in vivo toxic activities. These PT analogs, as well as S1(R9K,E129G)/S3(K82A) and S1(R9K,E129G)/S3(I91,Y92,K93) delta, still expressed an epitope which elicits a neutralizing antitoxin antibody and were protective in the mouse intracerebral challenge test. Recombinant pertussis vaccines based on PT analogs with **detoxifying**

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mutations in multiple subunits may thus represent the next generation of improved whooping cough vaccines.

L5 ANSWER 12 OF 19 TOXLINE

ACCESSION NUMBER: 1994:56416 TOXLINE

DOCUMENT NUMBER: CRISP-94-E00518-03

TITLE: DEVELOPMENT OF DETOXIFIED **PERTUSSIS**
TOXIN FOR ACELLULAR WHOOPING COUGH VACCINE.

AUTHOR: KEITH J M

CORPORATE SOURCE: NIDR, NIH

U.S. DEPT. OF HEALTH AND HUMAN SERVICES; PUBLIC
HEALTH SERVICE; NATIONAL INST. OF HEALTH, NATIONAL
INSTITUTE OF DENTAL RESEARCH.

CONTRACT NUMBER: Z01DE00518-03

SOURCE: (1992). Crisp Data Base National Institutes Of
Health. Award Type: G = Grant

DOCUMENT TYPE: (RESEARCH)

FILE SEGMENT: CRISP

LANGUAGE: English

ENTRY MONTH: 199403

AB RPROJ/CRISP Whooping cough is caused by an infection of the respiratory tract with *Bordetella pertussis* bacteria. This disease is effectively controlled by the current vaccine which consists of killed whole *B. pertussis* cells. Though efficacious, the present vaccine produces unacceptable side effects. The major protective antigen in whooping cough vaccines is **pertussis toxin**. Clinical trials of acellular pertussis products strongly indicate that **pertussis toxin** will be a necessary and perhaps sufficient component of any new vaccine. Chemically "inactivated" **pertussis toxin** vaccines have been produced with reduced side effects and reasonable efficacy, however, residual activity may exist. Through our gene expression experiments we discovered a molecular approach for inactivation of **pertussis toxin**. Using site-specific DNA mutagenesis, the S1 subunit was modified by either a single or double amino acid substitution. These mutations virtually eliminated toxic activity, yet the immunogenic protective epitope was retained. We have devised several methods to transfer these genetic changes into the chromosome of *B. pertussis*, thus creating several new mutant strains. Using these new **mutant** strains, a genetically **detoxified pertussis toxin** molecule has been produced. This nontoxic holotoxin has strong immunoprotective properties and can be used as a vaccine antigen without chemical inactivation. Immunoprotein studies as well as characterization of the biological activities associated with these new strains are currently underway in our laboratory and at the National Institute of Health in Tokyo, Japan. In addition to this effort, new constructs have been produced to utilize a live *Salmonella* oral vaccine. These construct are being tested in an

Searcher : Shears 308-4994

animal model as a collaboration with researchers at Washington University in St. Louis and University of Missouri in Columbia.

L5 ANSWER 13 OF 19 TOXLIT

ACCESSION NUMBER: 1992:98133 TOXLIT

DOCUMENT NUMBER: CA-117-144511Z

TITLE: Construction of Bordetella pertussis strains that overproduce genetically inactivated **pertussis toxin**.

AUTHOR: Zealey G; Loosmore S; Yacoob R; Cockle S; Herbert A; Klein M

CORPORATE SOURCE: Res. Connaught Lab., Connaught Cent. Biotechnol., Willowdale

SOURCE: Vaccines 92: Mod. Approaches New Vaccines Incl. Prev. AIDS [Annu. Meet.], 9th, (1992). pp. 367-71. CODEN: 57WXAL.

PUB. COUNTRY: Canada

DOCUMENT TYPE: Book; (MONOGRAPH)

FILE SEGMENT: CA

LANGUAGE: English

OTHER SOURCE: CA 117:144511

ENTRY MONTH: 199211

AB **Pertussis toxin (PT)** is a major protective antigen in pertussis vaccines. For max. immunogenicity and safety, **PT** should be detoxified by genetic rather than chem. means. Such detoxification has been achieved by site-directed mutagenesis of the tox operon and prodn. of nontoxic **PT** analogs by recombinant Bordetella pertussis strains (M. A. Pizza et al. 1989, S. M. Loosmore et al. 1990). One highly **detoxified PT** analog contains the **mutations** Arg-9.fwdarw.Lys and Glu-129.fwdarw.Gly in subunit S1. This mol. is immunogenic and protective and is an appropriate antigen for inclusion in an acellular whooping cough vaccine. However, the relatively low level of **PT** secretion by B. pertussis is a limiting factor in the prodn. of such analogs. To increase the secretion of the Lys9Gly129 **PT** analog by B. pertussis, addnl. copies of the mutated tox operon were integrated at the tox and fha loci by unmarked allelic exchange. The resulting recombinant strains secrete amts. of **PT** analog proportional to gene dosage, and yields of up to 80 mg/L can be obtained in 10-L fermentors.

L5 ANSWER 14 OF 19 BIOSIS COPYRIGHT 1999 BIOSIS

ACCESSION NUMBER: 1992:189143 BIOSIS

DOCUMENT NUMBER: BA93:100093

TITLE: EVALUATION OF ACELLULAR DPT VACCINES IN INFANTS.

AUTHOR(S): NENCIONI L; PODDA A; PEPPOLONI S; VOLPINI G; MARSILI I; CONTU B; COSSU M A; VANNI R; ET AL

CORPORATE SOURCE: R. RAPPUOLI, SCLAVO RES. CENT., VIA FIORENTIAN 1, Searcher : Shears 308-4994

09/044696

53100 SIENA, ITALY.
SOURCE: MEM INST BUTANTAN (SAO PAULO), (1991) 53 (SUPPL 1),
21-29.
CODEN: MIBUAH. ISSN: 0073-9901.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB Two acellular DPT vaccines containing, as pertussis components, the
genetically detoxified pertussis toxin
mutant PT-9K/129G, either alone or combined with
FHA and 69K, were evaluated for safety and immunogenicity in infants
8-14 months old. Both vaccines induced very mild local reactions
which were consistant with the presence of alum and the previous
administration of two doses of whole-cell DPT vaccine. A marked
increase in specific antibodies to each pertussis component and in
pertussis toxin neutralizing antibodies was
observed after one dose of either acellular vaccines. All vaccines
also acquired an excellent protective immunity against diptheria and
tetnus, as assessed in vitro and in vivo.

L5 ANSWER 15 OF 19 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 91034178 MEDLINE
DOCUMENT NUMBER: 91034178
TITLE: Engineering of genetically detoxified
pertussis toxin analogs for
development of a recombinant whooping cough vaccine.
AUTHOR: Loosmore S M; Zealey G R; Boux H A; Cockle S A;
Radika K; Fahim R E; Zobrist G J; Yacoob R K; Chong P
C; Yao F L; et al
CORPORATE SOURCE: Connaught Centre for Biotechnology Research,
Willowdale, Ontario, Canada..
SOURCE: INFECTION AND IMMUNITY, (1990 Nov) 58 (11) 3653-62.
Journal code: GO7. ISSN: 0019-9567.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199102

AB Pertussis toxin (PT) is an important
protective antigen in vaccines against whooping cough, and a
genetically detoxified PT analog is the preferred form of
the immunogen. Several amino acids of the S1 subunit were identified
as functionally critical residues by site-directed mutagenesis,
specifically, those at positions 9, 13, 26, 35, 41, 58, and 129.
Eighty-three mutated PT operons were introduced into
Bordetella parapertussis, and the resultant toxin analogs
were screened for expression levels, enzymatic activity, residual
toxicity, and antigenicity. While more than half of the mutants were
found to be poorly secreted or assembled, the rest were fully
assembled and most were highly detoxified. Single

Searcher : Shears 308-4994

mutations resulted in up to a 1,000-fold reduction in both toxic and enzymatic activities, while **PT** analogs with multiple mutations (Lys-9 Gly-129, Glu-58 Gly-129, and Lys-9 Glu-58 Gly-129) were 10(6)-fold detoxified. Operons coding for stable and nontoxic mutants shown to express a critical immunodominant protective epitope were returned to the chromosome of *Bordetella pertussis* by allelic exchange. In vivo analysis of the **toxin** analogs showed a dramatic reduction in histamine sensitization and lymphocytosis-promoting activities, paralleling the reduction in toxic activities. All mutants were protective in an intracerebral challenge test, and the Lys-9 Gly-129 analog was found to be significantly more immunogenic than the toxoid. **PT** analogs such as those described represent suitable components for the design of a recombinant whooping cough vaccine.

L5 ANSWER 16 OF 19 MEDLINE DUPLICATE 5

ACCESSION NUMBER: 91175025 MEDLINE
 DOCUMENT NUMBER: 91175025
 TITLE: Gene replacement in *Bordetella pertussis* by transformation with linear DNA.
 AUTHOR: Zealey G R; Loosmore S M; Yacoob R K; Cockle S A; Boux L J; Miller L D; Klein M H
 CORPORATE SOURCE: Connaught Centre for Biotechnology Research, Willowdale, Ontario, Canada..
 SOURCE: BIO/TECHNOLOGY, (1990 Nov) 8 (11) 1025-9. Journal code: AL1. ISSN: 0733-222X.
 PUB. COUNTRY: United States
 Journal; Article; (JOURNAL ARTICLE)
 LANGUAGE: English
 FILE SEGMENT: B
 ENTRY MONTH: 199107

AB We replaced the wild-type TOX operon of *Bordetella pertussis* with in vitro **mutated, detoxified** alleles by electroporetic transformation using unmarked linear DNA. Uptake of DNA was selected by transient ampicillin resistance and two simultaneous recombination events resulted in gene-replacement at the natural locus with no integration of heterologous DNA. TOX alleles were stable without selection and recombinant strains secreted non-toxic, fully assembled, protective **pertussis toxin (PT)** analogues with kinetics similar to the parental vaccine strain under production-scale fermentation conditions. Strains generated in this way are suitable for the production of recombinant whole-cell or component whooping cough vaccines that require no chemical modification of **PT**.

L5 ANSWER 17 OF 19 BIOSIS COPYRIGHT 1999 BIOSIS

ACCESSION NUMBER: 1990:365169 BIOSIS
 DOCUMENT NUMBER: BR39:49645
 TITLE: DETOXIFICATION OF PERTUSSIS
 Searcher : Shears 308-4994

09/044696

TOXIN BY MUTATIONS IN THE B
OLIGOMER GENE.

AUTHOR(S): LOCHT C; FERON C; DEQUESNE G; DE WILDE M
CORPORATE SOURCE: SMITH KLINE BIOLOGICALS, 89 RUE DE L'INST., B-1330
RIXENSART, BELG.
SOURCE: 90TH ANNUAL MEETING OF THE AMERICAN SOCIETY FOR
MICROBIOLOGY 1990, ANAHEIM, CALIFORNIA, USA, MAY
13-17, 1990. ABSTR ANNU MEET AM SOC MICROBIOL, (1990)
90 (0), 48.
CODEN: ASMACK. ISSN: 0094-8519.
DOCUMENT TYPE: Conference
FILE SEGMENT: BR; OLD
LANGUAGE: English

L5 ANSWER 18 OF 19 WPIDS COPYRIGHT 1999 DERWENT INFORMATION LTD
ACCESSION NUMBER: 1989-324080 [44] WPIDS
DOC. NO. NON-CPI: N89-246838
DOC. NO. CPI: C89-143525
TITLE: Vaccine contg. recombinant, detoxified Pasteurella
multocida toxin - to protect against organisms
producing osteolytic toxin.
DERWENT CLASS: B04 C03 D16 S03
INVENTOR(S): PETERSEN, S; TAEKKER, FOGED N; FOGED, N T; FOGED, T
N; TEKKER, F
PATENT ASSIGNEE(S): (INTE-N) INTERVET INT BV; (STAT-N) STATENS
VETERINAERE SERUMLABORATORIUM; (NDKE) NORDISK DROGE
& KEMIKALIE AS; (STAT-N) STATENS VETERIN SERUMLAB;
(VETE-N) STATENS VETERINAERE SERU
COUNTRY COUNT: 23
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 8909617	A	891019	(8944)*	EN	121
RW: AT BE CH DE FR GB IT LU NL SE					
W: AU BE BR DK FI HU JP KR NO RO SU US					
AU 8935320	A	891103	(9003)		
EP 409895	A	910130	(9105)		
R: AT BE CH DE FR GB IT LI LU NL SE					
DK 9002308	A	900924	(9106)		
EP 409895	B1	940622	(9424)	EN	78
R: AT BE CH DE FR GB IT LI LU NL SE					
DE 68916424	E	940728	(9429)		
US 5369019	A	941129	(9502)		61
DK 169749	B	950213	(9511)		
IE 64033	B	950628	(9533)		
US 5885589	A	990323	(9919)		

APPLICATION DETAILS:

Searcher : Shears 308-4994

09/044696

PATENT NO	KIND	APPLICATION	DATE
WO 8909617	A	WO 89-DK84	890411
EP 409895	A	EP 89-905073	890411
EP 409895	B1	EP 89-905073	890411
		WO 89-DK84	890411
DE 68916424	E	DE 89-616424	890411
		EP 89-905073	890411
		WO 89-DK84	890411
US 5369019	A	WO 89-DK84	890411
		US 90-582945	901012
DK 169749	B	WO 89-DK84	890411
		DK 90-2308	900924
IE 64033	B	IE 89-1151	890411
US 5885589	A Cont of	WO 89-DK84	890411
	Cont of	US 90-582945	901012
	Div ex	US 94-293314	940822
		US 95-453141	950530

FILING DETAILS:

PATENT NO	KIND	PATENT NO
EP 409895	B1 Based on	WO 8909617
DE 68916424	E Based on	EP 409895
	Based on	WO 8909617
US 5369019	A Based on	WO 8909617
DK 169749	B Previous Publ.	DK 9002308
US 5885589	A Cont of	US 5369019

PRIORITY APPLN. INFO: DK 88-1995 880412; DK 90-2308 900924

AN 1989-324080 [44] WPIDS

AB WO 8909617 A UPAB: 19950918

Vaccine for immunising animals or humans against diseases caused by microorganisms producing an osteolytic toxin, comprises recombinant, immunogenic, detoxified *Pasteurella multocida* (P.m.) toxin, or toxin analogue, plus an acceptable carrier or vehicle. Also new are (1) DNA fragments encoding P.m. toxin (or analogues); (2) expression vectors contg. these fragments; (3) microorganisms contg. such vectors, and (4) monoclonal antibodies MAb; and their fragments against P.m. toxin.

The toxin, or analogue, is detoxified by heating; chemical treatment, mutagenesis; or by substitution, deletion, addn. or insertion of at least one amino acid (or base pair in the corresponding nucleic acid coding sequences). The specification includes the DNA sequence (and derived amino acid sequence) which encodes for the toxin (4380 bases).

USE/ADVANTAGE - The vaccines are used to protect against esp.

Searcher : Shears 308-4994

P.m. (which causes progressive atrophic rhinitis in pigs) but also e.g. *Actinomyces viscosus* and *Bordetella pertussis*. Recombinant **toxins** can be produced without culturing pathogenic organisms and in improved yields.

1/33

Dwg.1/33

ABEQ EP 409895 B UPAB: 19940803

A DNA fragment encoding a *Pasteurella multocida* toxin comprising an amino acid sequence as shown in Fig. 10(a)-(j) (in the specification) or encoding an immunogenic subsequence or analogue of said toxin.

Dwg.0/22

ABEQ US 5369019 A UPAB: 19950117

Recombinant DNA encodes the prodn. of a *Pasteurella multocida* toxin polypeptide. The nucleotide sequence of the cDNA and the amino acid sequence of the polypeptide are defined.

Plasmids and expression vectors contg. the DNA are new. Host cells (e.g. *Escherichia coli*) are transformed with the plasmids and vectors and then propagated to produce and then reacted with HCHO or glutaraldehyde, or subjected to proteolytic enzymolysis, to give derivs. with much reduced toxicity.

USE/ADVANTAGE - The detoxified polypeptide derivs. are dispersed with the usual carriers and additives to provide vaccines against disease caused by the osteolytic *Pasteurella multocida* toxin. The vaccine is effective against porcine atrophic rhinitis.

Dwg.0/0

L5 ANSWER 19 OF 19 WPIDS COPYRIGHT 1999 DERWENT INFORMATION LTD

ACCESSION NUMBER: 1989-186481 [26] WPIDS

CROSS REFERENCE: 96-041582 [05]

DOC. NO. CPI: C89-082452

TITLE: Immuno-protective, genetically-detoxified
pertussis toxin and vaccine -
with aminoacid substitution(s) or deletion(s)
produced by site-directed mutagenesis of toxin
gene.

DERWENT CLASS: B04 D16

INVENTOR(S): BOUX, H A; COCKLE, S A; KLEIN, M H; LOOSMORE, S M;
ZEALEY, G R

PATENT ASSIGNEE(S): (CONN-N) CONNAUGHT LABS LTD; (CONN-N) CONNAUGHT LAB
LTD

COUNTRY COUNT: 15

PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
EP 322115	A	890628	(8926)*	EN	42
R: AT BE CH DE ES FR GB GR IT LI LU NL SE					
JP 02002383	A	900108	(9007)		

Searcher : Shears 308-4994

09/044696

US 5085862 A 920204 (9208) 33
 US 5221618 A 930622 (9326) 37
 US 5244657 A 930914 (9338) 46
 US 5332583 A 940726 (9429) 45
 US 5358868 A 941025 (9442) 45
 US 5433945 A 950718 (9534) 47
 EP 322115 B1 960306 (9614) EN 49
 R: AT BE CH DE ES FR GB GR IT LI LU NL SE
 DE 3855072 G 960411 (9620)
 ES 2088778 T3 960916 (9643)
 JP 2714068 B2 980216 (9812) 37

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
EP 322115	A	EP 88-311133	881124
JP 02002383	A	JP 88-297152	881124
US 5085862	A	US 88-275376	881123
US 5221618	A Div ex	US 88-275376	881123
		US 91-767837	910930
US 5244657	A CIP of	US 88-275376	881123
		US 90-589423	900928
US 5332583	A CIP of	US 88-275376	881123
	Div ex	US 89-589423	890928
		US 91-788314	911105
US 5358868	A CIP of	US 88-275376	881123
	Div ex	US 90-589423	900928
		US 91-788313	911105
US 5433945	A CIP of	US 88-275376	881123
	Div ex	US 90-589423	900928
		US 92-979798	921120
EP 322115	B1	EP 88-311133	881124
DE 3855072	G	DE 88-3855072	881124
		EP 88-311133	881124
ES 2088778	T3	EP 88-311133	881124
JP 2714068	B2	JP 88-297152	881124

FILING DETAILS:

PATENT NO	KIND	PATENT NO
US 5221618	A Div ex	US 5085862
US 5244657	A CIP of	US 5085862
US 5332583	A CIP of	US 5085862
	Div ex	US 5244657
US 5358868	A CIP of	US 5085862
	Div ex	US 5244657
US 5433945	A CIP of	US 5045862

Searcher : Shears 308-4994

	Div ex	US 5244657
DE 3855072	G Based on	EP 322115
ES 2088778	T3 Based on	EP 322115
JP 2714068	B2 Previous Publ.	JP 02002383

PRIORITY APPLN. INFO: GB 87-27489 871124

AN 1989-186481 [26] WPIDS

CR 96-041582 [05]

AB EP 322115 A UPAB: 19960212

An immunoprotective, genetically-detoxified mutant of **pertussis toxin** is new. Vaccine against *Bordetella pertussis* comprises an effective amt. of the mutant, or its toxoid, and an acceptable carrier. Conjugate vaccine comprises the mutant as carrier protein for a hapten, polysaccharide or polypeptide. New strains of *Bordetella pertussis* are characterised by either (i) the absence of the toxin operon and foreign DNA and by the ability to be grown in the absence of antibiotics to produce B. *pertussis* antigens free of **pertussis toxin**; or (ii) the toxin operon having been replaced by a mutant gene formed by site-directed mutagenesis of at least one specific amino acid residue responsible for **pertussis toxin** toxicity. Native *Bordetella pertussis* 10536 TOX operon is new having a given nucleotide sequence and structural gene translation.

ADVANTAGE - Residual toxicity is 1% or less, pref. less than 0.5% of that of the native toxin. Genetic detoxification avoids the problems of chemical detoxification using e.g. formaldehyde, glutaraldehyde or H₂O₂, i.e. obtaining a balance between sufficient detoxification and loss of potency.

0/20

Dwg.0/20

ABEQ US 5085862 A UPAB: 19930923

Immunoprotective genetically detoxified mutant of **pertussis holotoxin** is formed by genetic modification of the A portion (S1 subunit) and/or B portion of the holotoxin.

Pref. a single amino acid in the native holotoxin is removed or replaced e.g. glu-129 is removed and opt. replaced by gly, or arg-58 is replaced by glu, etc. Mutant has residual toxicity, less than 0.5% of native toxin.

ADVANTAGE - Has decreased histamine sensitivity in a vaccine against *Bordetella pertussis*.

ABEQ US 5221618 A UPAB: 19931116

Strain of *Bordetella* capable of expressing an immunoprotective genetically-detoxified mutant of **pertussis holotoxin**. Toxin operon has been replaced by a mutant operon formed by mutagenesis of a nucleotide sequence encoding at least one specific amino acid residue which contributes to **pertussis toxin** toxicity.

Also claimed is a method of producing an immunoprotective, genetically-detoxified **pertussis holotoxin mutant**

Searcher : Shears 308-4994

USE/ADVANTAGE - As a vaccine against pertussis.

Dwg.0/10

ABEQ US 5244657 A UPAB: 19931123

Immunoprotective genetically-detoxified mutant of **pertussis** halo-toxin has a single amino acid in its S1 sub-unit of the native form replaced, i.e. arg9 by lys9.

Mutant has residual toxicity less than 0.5% of native toxic. Prodn. comprises site-directed mutagenesis of native **pertussis toxin** gene. Mutant has decreased histamine sensitivity activity.

USE - In prepn. of safe, immunogenic and efficacious vaccine for protection against pertussis.

Dwg.0/15

ABEQ US 5332583 A UPAB: 19940907

Vaccine against *Bordetella pertussis* comprises a mutant of pertussis holotoxin (where at least one amino acid is removed or replaced) and at least one other pertussis antigen e.g. agglutinogens, FHA or 69 kD membrane protein.

ADVANTAGE - Vaccine is safe and effective.

Dwg.0/29

ABEQ US 5358868 A UPAB: 19941212

Strain of *Bordetella* has the toxin operon replaced by a mutant gene formed by site-directed mutagenesis of a sequence encoding the S1 and S3 subunit of pertussis holotoxin. Has ATCC Nos. 53833, 53834, 53836, 53837, 53974, 53975 or 53976.

USE/ADVANTAGE - Prepn. of a vaccine against pertussis. Vaccine is safe.

Dwg.0/29

ABEQ US 5433945 A UPAB: 19950904

Immunoprotective genetically-detoxified mutant of pertussis holotoxin has multiple amino acids in the native toxin replaced or removed. Specific examples include Arg-58 and Gly-129 replaced by Glu-58 and Gly-129, and Arg-9 and Glu-129 replaced by Ly's-9 and Gly-129 in the SI subunit. Mutants have a residual toxicity of less than 0.5%.

USE/ADVANTAGE - Used as a vaccine against pertussis. Retains immunological properties without having undesirable side effects. decreased histamine sensitivity.

Dwg.0/15

ABEQ EP 322115 B UPAB: 19960405

A mutant pertussis holotoxin obtained by expression of a tox operon encoding the holotoxin which has been mutated by site-directed mutagenesis of at least one codon encoding at least one functional amino acid within native pertussis holotoxin including at least one of (A1) ARG9, ARG13 and GLU129, to effect removal or replacement of said at least one functional amino acid and to genetically detoxify said holotoxin to a residual toxicity of 1% or less while retaining immunoprotective properties.

Searcher : Shears 308-4994

Dwg.0/10

=> d his 16-; d 1-6 ibib abs

(FILE 'CAPLUS, MEDLINE, BIOSIS, EMBASE, TOXLIT, TOXLINE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, CABA, AGRICOLA' ENTERED AT 15:23:45 ON 27 JUL 1999)

L6 74 S BARCHFELD G?/AU
 L7 580 S (DELGIUDICE G? OR DEL GIUDICE G?)/AU
 L8 1744 S RAPPUOLI R?/AU
 L9 2 S L6 AND L7 AND L8
 L10 7 S L6 AND (L7 OR L8)
 L11 46 S L7 AND L8
 L12 7 S L11 AND L1
 L13 12 S L9 OR L10 OR L12
 L14 6 DUP REM L13 (6 DUPLICATES REMOVED)

-Author(s)

L14 ANSWER 1 OF 6 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 1
 ACCESSION NUMBER: 1999:164424 CAPLUS
 DOCUMENT NUMBER: 131:17638
 TITLE: The adjuvants MF59 and LT-K63 enhance the mucosal and systemic immunogenicity of subunit influenza vaccine administered intranasally in mice
 AUTHOR(S): Barchfeld, G. L.; Hessler, A. L.; Chen, M.; Pizza, M.; Rappuoli, R.; Van Nest, G. A.
 CORPORATE SOURCE: Chiron Corporation, Emeryville, CA, 94608-2916, USA
 SOURCE: Vaccine (1999), 17(7-8), 695-704
 CODEN: VACCDE; ISSN: 0264-410X
 PUBLISHER: Elsevier Science Ltd.
 DOCUMENT TYPE: Journal
 LANGUAGE: English

AB Com. influenza vaccines generate serum antibody, but not local IgA. Influenza vaccines that induce both serum and secretory antibody are more likely to protect against infection and disease progression. The adjuvants MF59 and LT-K63 were tested i.m. and intranasally with subunit HA. In naive mice, intranasal adjuvant effect was more apparent when included with the first than second immunization. In previously infected mice, intranasal adjuvants had little effect on serum antibodies and were most effective for nasal antibodies after the second immunization. Overall, both adjuvants enhanced anti-HA IgA and IgG by intranasal vaccination whereas, by i.m. vaccination, they only enhanced serum IgG.

L14 ANSWER 2 OF 6 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 2
 ACCESSION NUMBER: 1998:672490 CAPLUS
 DOCUMENT NUMBER: 129:289177
 Searcher : Shears 308-4994

TITLE: Detoxified mutants of bacterial ADP-
ribosylating toxins as
parenteral adjuvants
INVENTOR(S): Barchfeld, Gail; Del Giudice,
Giuseppe; Rappuoli, Rino
PATENT ASSIGNEE(S): Chiron Corporation, USA
SOURCE: PCT Int. Appl., 51 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
-----	---	-----	-----	-----
WO 9842375	A1	19981001	WO 98-US5454	19980319
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9865713	A1	19981020	AU 98-65713	19980319
PRIORITY APPLN. INFO.:			US 97-41227	19970321
			US 98-44696	19980318
			WO 98-US5454	19980319
AB The present invention provides parenteral adjuvants comprising detoxified mutants of bacterial ADP-ribosylating toxins , esp. pertussis toxin (PT)), cholera toxin (CT) , and <i>Escherichia</i> coli -derived heat-labile toxin (LT) . The immune adjuvant includes LT-K63, LT-R72, CT-S109 and PT-K9/G129. LT-K63 was prepd. as parenteral adjuvant for vaccine comprising herpes simplex virus type 2 gD antigen, influenza hemagglutinin, and HIV p24 gag.				

L14 ANSWER 3 OF 6 SCISEARCH COPYRIGHT 1999 ISI (R)

ACCESSION NUMBER: 1998:308436 SCISEARCH

THE GENUINE ARTICLE: ZH131

TITLE: Mucosal adjuvanticity and immunogenicity of LTR72, a
novel mutant of *Escherichia coli* heat-labile
enterotoxin with partial knockout of
ADP-ribosyltransferase activity
AUTHOR: Giuliani M M; DelGiudice G; Giannelli V;
Dougan G; Douce G; Rappuoli R (Reprint);
Pizza M

Searcher : Shears 308-4994

CORPORATE SOURCE: CHIRON SPA, IRIS, VIA FIORENTINA 1, I-53100 SIENA, ITALY (Reprint); CHIRON SPA, IRIS, I-53100 SIENA, ITALY; UNIV LONDON IMPERIAL COLL SCI TECHNOL & MED, DEPT BIOCHEM, LONDON SW7 2AY, ENGLAND

COUNTRY OF AUTHOR: ITALY; ENGLAND

SOURCE: JOURNAL OF EXPERIMENTAL MEDICINE, (6 APR 1998) Vol. 187, No. 7, pp. 1123-1132.
 Publisher: ROCKEFELLER UNIV PRESS, 1114 FIRST AVE, 4TH FL, NEW YORK, NY 10021.
 ISSN: 0022-1007.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE

LANGUAGE: English

REFERENCE COUNT: 58

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Heat-labile Escherichia coli enterotoxin (LT) has the innate property of being a strong mucosal immunogen and adjuvant. In the attempt to reduce toxicity and maintain the useful immunological properties, several LT mutants have been produced. Some of these are promising mucosal adjuvants. However, so far, only those that were still toxic maintained full adjuvanticity. In this paper we describe a novel LT mutant with greatly reduced toxicity that maintains most of the adjuvanticity. The new mutant (LTR72), that contains a substitution Ala --> Arg in position 72 of the A subunit, showed only 0.60% of the LT enzymatic activity, was 100,000-fold less toxic than wild-type LT in Y1 cells in vitro, and was at least 20 times less effective than wild-type LT in the rabbit ileal loop assay in vivo. At a dose of 1 mu g, LTR72 exhibited a mucosal adjuvanticity, similar to that observed with wild-type LT, better than that induced by the nontoxic, enzymatically inactive LTK63 mutant, and much greater than that of the recombinant B subunit. This trend was consistent for both the amounts and kinetics of the antibody induced, and priming of antigen-specific T lymphocytes. The data suggest that the innate high adjuvanticity of LT derives from the independent contribution of the nontoxic AB complex and the enzymatic activity. LTR72 optimizes the use of both properties: the enzymatic activity for which traces are enough, and the nontoxic AB complex, the effect of which is dose dependent. In fact, in dose-response experiments in mice, 20 mu g of LTR72 were a stronger mucosal adjuvant than wild-types LT. This suggests LTR72 may be an excellent candidate to be tested in clinical trials.

L14 ANSWER 4 OF 6 CAPLUS COPYRIGHT 1999 ACS

ACCESSION NUMBER: 1998:673563 CAPLUS

DOCUMENT NUMBER: 130:79917

TITLE: LT and CT mutants as mucosal adjuvants

AUTHOR(S): Del Giudice, Giuseppe; Pizza, Mariagrazia; Rappuoli, Rino

CORPORATE SOURCE: Chiron SpA Res. Cent., IRIS, Siena, 53100, Italy

Searcher : Shears 308-4994

09/044696

SOURCE: Mol. Aspects Med. (1998), 19(1), 37-46, 47-70
CODEN: MAMED5; ISSN: 0098-2997
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review with approx. 275 refs. about cholera toxin and Escherichia coli heat-labile toxin as mucosal adjuvants and immunogens and their possible use a mucosally delivered vaccines.

L14 ANSWER 5 OF 6 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1998:673548 CAPLUS
DOCUMENT NUMBER: 130:79916
TITLE: PT mutants as vaccines against pertussis
AUTHOR(S): Del Giudice, Giuseppe; Pizza, Mariagrazia; Rappuoli, Rino
CORPORATE SOURCE: Chiron SpA Res. Cent., IRIS, Siena, 53100, Italy
SOURCE: Mol. Aspects Med. (1998), 19(1), 27-36, 47-70
CODEN: MAMED5; ISSN: 0098-2997
PUBLISHER: Elsevier Science Ltd.
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English
AB A review with more than 100 refs. Topics discussed include whole cell pertussis vaccine; B. pertussis antigens for acellular pertussis vaccines; genetically detoxified acellular pertussis vaccines; improved immunogenicity and efficacy of genetically detoxified recombinant pertussis vaccine;.

L14 ANSWER 6 OF 6 CAPLUS COPYRIGHT 1999 ACS DUPLICATE 3
ACCESSION NUMBER: 1998:419520 CAPLUS
DOCUMENT NUMBER: 129:197743
TITLE: Immunogenicity and adjuvantcity of partially or completely detoxified LT derivatives
AUTHOR(S): Giuliani, M. M.; Del Giudice, G.; Douce, G.; Dougan, G.; Rappuoli, R.; Pizza, M.
CORPORATE SOURCE: IRIS, Chiron Vaccines Immunobiological Research Institute in Siena, Italy
SOURCE: Zentralbl. Bakteriол., Suppl. (1997), 29(Bacterial Protein Toxins), 458-460
CODEN: ZBASE2; ISSN: 0941-018X
PUBLISHER: Gustav Fischer Verlag
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Heat-labile enterotoxin (LT) from the enterotoxigenic Escherichia coli is an ADP-ribosylating toxin. It is a strong immunogen, but its toxicity has precluded its use in the mucosally delivered vaccines. Here, the authors describe a new mutant in the A subunit of LT, LTR72 (Ala72
Searcher : Shears 308-4994

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to Arg) which retains a residual but very low toxicity. They compare the immunogenicity and adjuvanticity of LTR72 with those of the fully active wild-type LT, the non-toxic LTK63 mutant, and the recombinant LTB to exploit the role of the A subunit and of ADP-ribosylating activity on mucosal immunogenicity and adjuvanticity.

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